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CONSERVATION AND PROTECTION
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NORTH VANCOUVER, B.C.

STANDARD LEACHING TEST FOR ANTISAPSTAIN CHEMICALS

Regional Program Report 90-10

BY

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EXECUTIVE SUMMARY

Research conducted by various government and private laboratories showed that antisapstain chemicals will leach from treated lumber. Various studies have been done to simulate this leaching in laboratory scale tests. Non-standard test conditions such as variations in lumber package sizes, intensity and duration of artificial rainfall and sampling frequency have made it difficult to relate the results between tests and to conditions which would normally be experienced by operating antisapstain facilities.

Recent leaching studies conducted at various mills by Environment Canada have indicated that leachate dripping from treated lumber is diluted an average of 15 times by rainfall before it discharges from the storage yard. Numerous variables at individual mill sites caused a range of dilutions from 6 to 24 times. These observations led to the development of a standard leaching test which can be conducted using natural or artificial rain. The standard leaching test can be used to predict the concentrations of the antisapstain chemicals in the runoff at operating facilities. Environment Canada has recommended to Agriculture Canada that the test procedure be included in the registration requirements for new antisapstain formulations.

SOMMAIRE

Les travaux de recherche effectues dans divers laboratoires du gouvernement et de l'entreprise privee ont revele que les produits de lutte contre les taches de l'aubier ont tendance a s'ecouler hors de bois traite sous l'action de la pluie. Divers essais de simulation en laboratoire ont ete executes mais comme ils ont ete faits independamment de toute norme (dimensions des eprouvettes, intensite et duree de la pluie artificielle utilisee et frequence de echantillonnages), il est tres difficile de comparer leurs resultats et d'etablir une correlation avec les conditions d'exploitation reelles.

Les etudes recemment effectuees par Environnement Canada dans diverses scieries indequent que la pluie dilue de quinze fois le degre de concentration des produits de traitement s'ecoulant du bois avant que ceux-ci n'aient evacue l'aire d'entreposage. Precisons toutefois que les variables d'un site a liautre etaient telles que les valeurs de degre de dilution obtenues se situaient a l'interieur d'une fourchette de 6 a 24. Pour reduire cet ecart, les chercheurs ont mis au point d'une methode d'essai standard pouvant etre executee avec de la pluie naturelle ou de la pluie artificielle et grace a laquelle il est possible de determiner le degre de concentration des produits de traitement contre les taches de l'aubier presents dans les eaux de ruissellement des scieries. Environnement Canada a recommande que cet essai fasse partie des criteres d'homologation des nouveaux produits de traitement contre les taches de l'aubier.

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1.0 OBJECTIVES

The objectives of this report are:

(1) to provide a standard method to determine the concentration of antisapstain chemical in rainwater leachate dripping from treated lumber;

(2) to provide a standard method to determine how the leachability of Resin Acids from lumber is affected by the application of antisapstain chemical; and

(3) to provide a standard method to predict the concentration of antisapstain chemical and resin acids in rainwater runoff from a treated lumber storage yard.

1.1 Introduction

Softwood lumber (other than cedar) is susceptible to attack by sapstain and mold fungi that cause stains and blemishes, which reduce the marketability of the lumber. The growth of these fungi can promote the invasion of organisms which can cause structural damage to the wood. To protect freshly cut lumber it is treated with chemical fungicides (antisapstains) prior to export. These fungicides are easily leached from wood on exposure to rainfall.

Sawmills and lumber export terminals which treat and/or store lumber treated with antisapstain chemicals are subject to precipitation in various forms. In British Columbia these facilities may be subject to annual precipitation which ranges from several millimetres in interior regions to over 2400 millimetres in coastal areas.(1)

The areas dedicated to the storage of treated lumber may range from 0.4 hectares to over 32 hectares (1.0 to 80 acres). These storage yards may contain up to 142,000 m^3 (60,000,000 board feet) of lumber which may have up to 7,000,000 m^2 (75,000,000 ft^2) of treated wood surface.

Rainfall on these large lumber storage areas can generate large volumes of contaminated yard runoff. the volumes of runoff for sawmills can exceed 400,000 litres per day and for large export terminals the volume can exceed 1,300,000 litres per day. This runoff can form plumes in receiving waters which are toxic to fish and other organisms.(1,2,3)

Some antisapstain formulations contain solvents or emulsifiers, which may enhance the leaching of resin acids from treated lumber.(6) Resin acids are toxic to fish and may add to the toxicity of the storage yard runoff.(7) Failure to account for the toxicity of resin acids in lumber leachate may result in a greater toxicity being attributed to the antisapstain chemical. It is therefore important to compare the concentration of antisapstain chemicals and resin acids in leachate from the control packages with leachate from packages treated with antisapstain chemicals.

The release of antisapstain chemicals in yard runoff is controlled by both federal and provincial legislation in British Columbia. The B.C. Provincial Waste Management Act, "Antisapstain Chemical Waste Control Regulation", specifically limits the concentrations of antisapstain chemicals in runoff from treated lumber storage yards.(5) The federal Fisheries Act in Section 36.(3) states that "no person shall deposit or permit the deposit of a deleterious substance of any type in water frequented by fish...".(4)

Laboratory studies have shown that antisapstain chemicals will leach from treated wood. It has been difficult to compare the results of various laboratory studies or to relate them to observations in the "real world" due to the wide variations in test protocol. Field studies and legal investigations have shown that the runoff from lumber storage yards would violate both the Fisheries Act and the provincial regulations.

There is a need to have a standardized procedure for the testing of the leachability of antisapstain chemicals from treated lumber to provide consistent, comparable results, which can be correlated to actual storage yard conditions. The method shown in this report is recommended to provided a reasonable prediction of the concentration of antisapstain chemical in the runoff discharged from a treated lumber storage yard. The method assumes that the treatment system and storage yard will conform to all the provisions required in the "Antisapstain Chemical Waste Control Regulation".(5)

1.2 Variables That Will Affect the Concentration of Antisapstain Chemical in Yard Runoff

The concentrations of antisapstain chemical in runoff will be affected by numerous variables including but not limited to:

1. Antisapstain chemical formulation
2. Chemical retention by the wood (application rate)
3. Lumber cut (rough vs. planed)
4. Lumber packaging and stacking
5. Drying time prior to exposure to precipitation
6. Precipitation duration
7. Precipitation intensity
8. Precipitation frequency
9. Precipitation pH
10. Quantity of treated lumber on the storage site
11. Species of lumber treated
12. General house keeping practices
13. 1st, 2nd or 3rd growth lumber
14. Solubility of the chemical in water
15. Diffusion of the chemical into the wood
16. Additives in the formulations
17. Exposure and degradation due to ultraviolet light
18. Microbial action
19. Ambient temperatures
20. Affinity to soils to yard surfaces

Based on results of other tests it appears that the most critical variables are, chemical retention, precipitation intensity (dilution) and quantity of treated wood on the storage site.

1.2.1 Antisapstain Chemical Formulation and Application Method.

This leaching test method shall assume that:

- the antisapstain chemical is formulated to provide a minimum of one years protection against sapstain fungi and molds under conditions which are application to the British Columbia climate and lumber species;

- the chemical application method will comply with the manufacturers recommended dosage of active ingredient per unit area of wood surface and that all exposed surfaces of the wood will be so treated;
- the covered storage time after treatment and prior to exposure to real or simulated precipitation will comply with the British Columbia, "Antisapstain Chemical Waste Control Regulation", and the antisapstain chemical manufacturers recommendations. (See Appendix I)

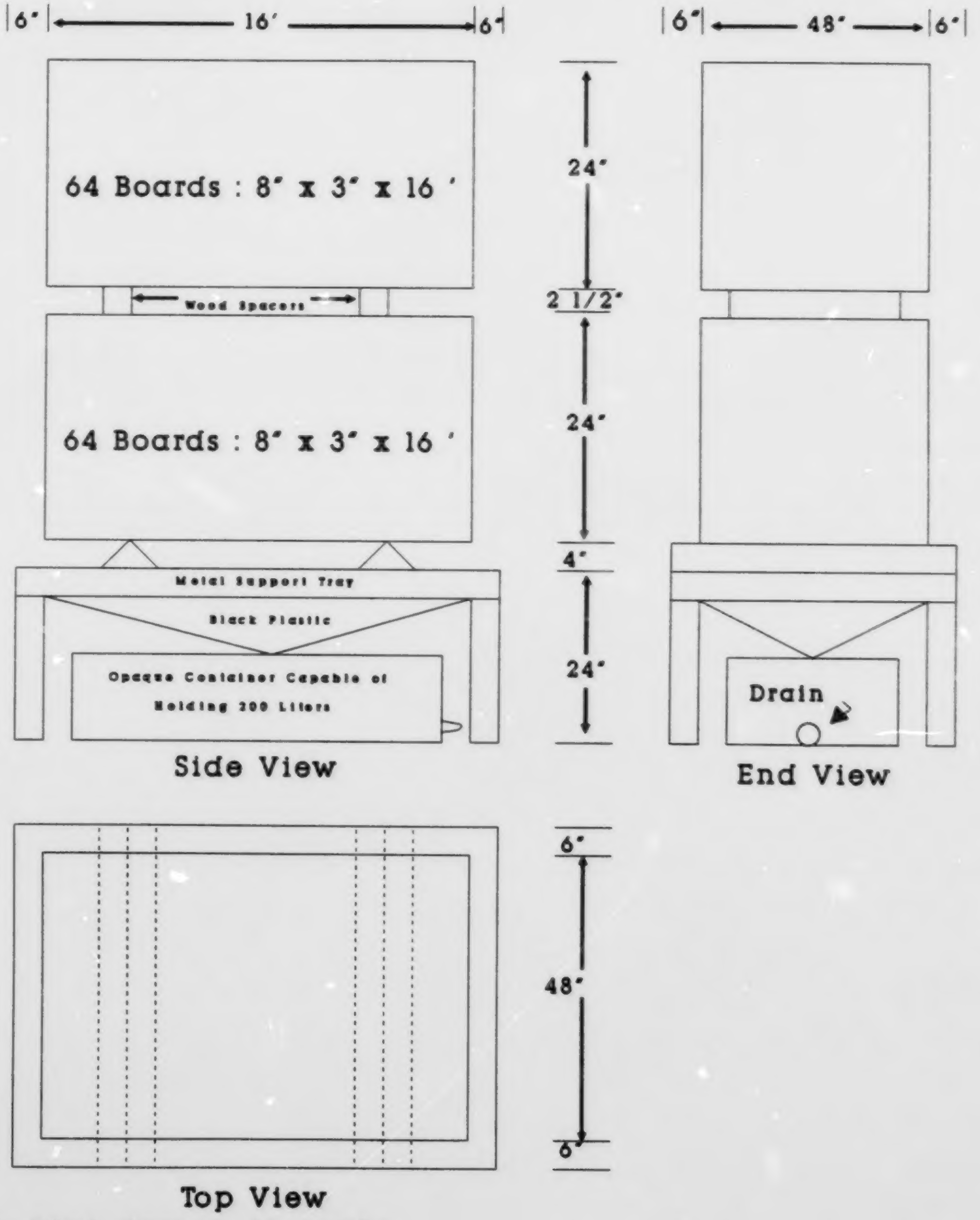
1.2.2 **Standard Test Packages and Test Loading.** The following criteria are recommended for testing. If variations are made to these procedures they must be accounted for in the final report of the test data. If smaller scale tests in terms of lumber package sizes are used, then the collection trays should be scaled accordingly. It is recommended that the precipitation rate not exceed 3.0 mm/h regardless of the test size of the lumber package as dilution effects will make correlation to "real world" situations very difficult.

A standard lumber package should be comprised of a wood species for which the antisapstain chemical is known to provide effective protection against the target molds and fungi for a period of not less than one year. The species of wood used for the leaching test must be reported in the test results.

In lumber storage yards, the packages are stacked from four to eight packages high. This allows leachate from one package to cascade to another with the potential to increase the concentration as well as increase the amount of liquid absorbed by the lumber. It is difficult, costly and probably unsafe to use test stacks of this height. To account for this cascade and absorption effect a minimum of two standard packages is recommended.

Two standard packages will be stacked one on top of the other with 6.4 cm (2.5 in) wood spacers separating the packages and centered on a standard leachate collection tray. (See Figure 1)

Figure #1: Standard Leaching Test Apparatus for Antisapstain Chemicals



Note: Not drawn to scale

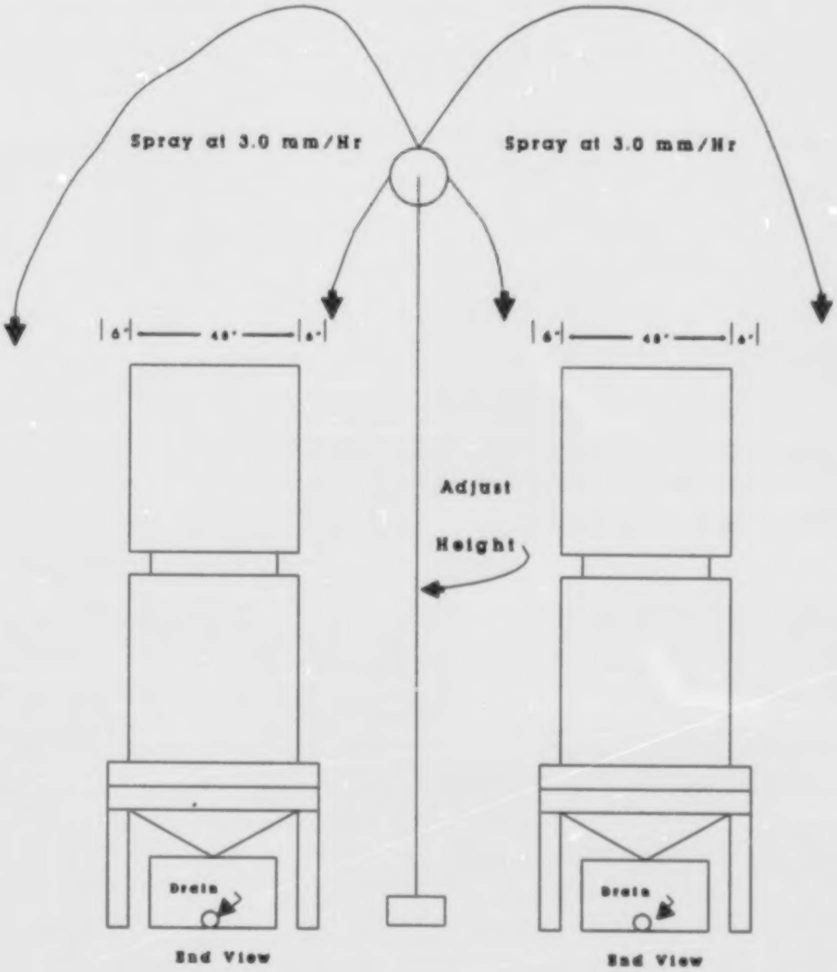
Note: The width and height and length of lumber package used should be 0.61 m x 1.22 m x 4.88 m (24 in. high x 48 in. wide x 16 ft. long). Lumber mills that wish to modify this test to suit their production format should choose the dimensions of the board that matches the largest proportion of the storage yard inventory. The results must note the changes in the dimensions of lumber used and the size of the packages formed.

The standard leachate tray is designed to allow tests using plastic/ paper caps or barrier layers under the top tier of the wood. This will allow for 5.0 to 7.6 cm (2 in. to 3 in.) eaves which extend beyond the perimeter of the package and shed water away from the wood. The plastic/paper layers are designed to prevent water from percolating through the packages and the eaves are designed to reduce or prevent the cascading of water from the wood of one package to another. If the trays were shorter or narrower, rainwater and/or leachate dripping from the eaves would fall outside the collection tray. This would make it difficult to compare the results to control packages, which did not have the caps or barrier layers.

The standard leachate collection tray should have a leachate collection surface area measuring 1.52 m x 5.2 m (5 ft. x 17 ft.). The collection surface should have a support structure of sufficient strength to support the weight of at least four standard packages. The support structure will have a minimum of 0.61 m (24 in.) of clearance between the ground and the leachate collection surface. The support structure should have 2.5 cm to 5.0 cm (1.0 to 2.0 in.) of slope over the 5.2 m (17 ft.) length of the tray to simulate the actual slope on a typical mill yard (actual slope must be reported in the analytical results). The tray should be levelled across the 1.52 m (5 ft.) width.

The surface of the leachate collection tray should be made of black polyethylene plastic, which covers the entire leachate collection surface and drains to a single drainage hole. (See plates 1,2,3,4) The untreated control lumber and the treated lumber should be subjected to the same rainfall. The recommended configuration is illustrated in Figure 2. The artificial rain of

Figure 2: Layout of Equipment to Simulate Leaching by Natural Rainfall



3 mm/h can be achieved by elevating the spray nozzle and controlling the water flow. The precipitation is measured by using a commercially available rainguage. The rainguage should be mounted so that the open collection orifice is at or just above the level of the top of the lumber.

1.2.3. Precipitation Intensity, Duration and Frequency. Over fifty percent of antisapstain facilities in British Columbia experience extremes in annual precipitation found at Vancouver Airport and Squamish, B.C. Over the past 80 years, Vancouver Airport has recorded an annual average of 163 days of measurable rainfall at 6.4 mm per day for an annual total of 1112 mm. Squamish has recorded an average of 165 days of measurable rainfall at 12.6 mm per day for an annual total of 2247 mm. The months of October, November, December, and January have the highest average rainfall days and are considered the worst case months for leaching of antisapstain chemicals. For these months the average daily accumulation is 8.1 mm for Vancouver Airport and 17.7 mm for Squamish. The average value for these areas combined is then 12.9 mm. The average number of days of rain for these regions during this period is 18.4 (18.3 for Vancouver Airport and 18.5 for Squamish).

The average worst case month thus has a monthly accumulation of 238 mm of rain. If artificial rain is to be used for simulated leaching it would have to be applied every other day for a total of 16 leaching cycles at a rate of 15 mm/day. the duration of rain would be 5 hours long with a target intensity of 3 mm per hour. This would total 240 mm of rain over a one month period.

An examination of data for leaching trials involving chlorophenols, TCMTB, borates and DDAC showed that within 7 leaching cycles (or 105 mm of rain) the concentration of antisapstain in the leachate approached a linearly decaying curve.(9,10,11,12, Figure 3). A leaching test is recommended to have at least 8 leaching cycles of 5 hours duration at a rate of 3 mm/hr to establish the leaching characteristics of a particular antisapstain chemical. Estimates of the leaching characteristics over a full 16 cycles may be estimated by extrapolation of the linear portion of the curve over the full 16 cycles.

Figure 3: Concentrations of Various Antisapstains in Leachate over Multiple Rainfall Cycles

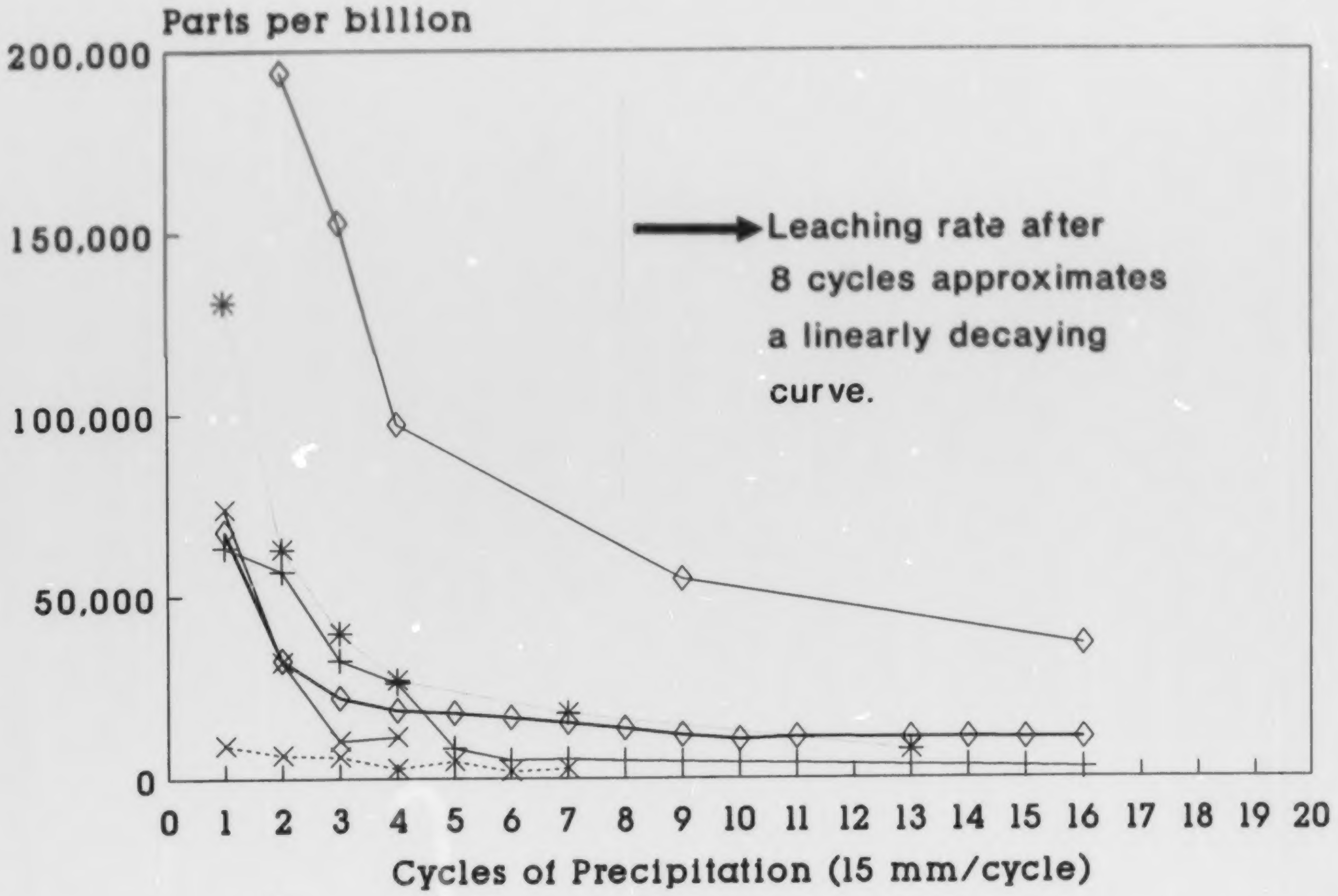


Plate #1: (left) Black polyethylene tarp is attached to the top of the support structure to form the leachate collection tray.

Plate #2: (Below) A single hole is made at the lowest center point at the downslope end of the tray.

Plate #3: Side view of two standard test packages loaded on the leachate collection tray. Arrow shows leachate collection jar.

Plate #4: End view of Two standard bundles loaded on a leachate collection tray

Days 1(15 mm), 3(30 mm), 5(45 mm), 7(60 mm), 9(75 mm), 11(90 mm), 13(105 mm), 15(120 mm) are considered benchmark sample collection days. Bracketed values indicate benchmark rainfall accumulations. The maximum volume of leachate collected per leaching cycle will be 118 L.

If there is a need to have an accelerated leaching test the duration of the rainfall should be increased to provide for more leaching action per cycle. The intensity of the rainfall should be maintained at 3.0 mm/h. For example, if a test is required to be completed within 4 cycles there should be 4 rainfall cycles at 3.0 mm/h for 10 hours. Using higher rates of rainfall will significantly increase dilution and impair the correlation to actual leaching conditions.

The leachate collection tray should be drained and rinsed after each benchmark accumulation has been sampled. A new plastic liner should be installed before the test is resumed.

If the test is subject to natural precipitation in addition to artificial rain the total rainfall per day should be recorded. If the natural rainfall occurs on an even or "dry day" the accumulation should be recorded and the next "artificial" rain should be applied as scheduled. Samples should be collected as close to the benchmark accumulations as possible taking into account both natural and artificial rain. The test may be ended when 120 mm of rain has accumulated.

Any changes to the test method must be described in the final report.

In tests conducted by Environment Canada, two sprinkler systems provided the target precipitation rate: - The "Gardinia Spray/sprinkler system, Type: Soft Rose Spray rotating head. The nozzle of this sprinkler was modified using a fine wire mesh to diffuse the spray pattern. The nozzle was mounted on a telescoping support stand which raised the nozzle 5.5 m (18 ft.) above ground level to achieve 3.0 mm/hr.

- The "Toro 300" series, "Stream Rotor" sprinkler using nozzle model #63-low gallonage elevated 5.5 m above ground level. This model was able to achieve a deposition rate of 2 mm/h to 4 mm/h without modification.

Several calibration tests were required to adjust water pressure at the nozzle to give a widely diffused pattern which simulated natural rain with a deposition rate of 3 mm/h. Location of the test site in an area devoid of strong cross winds will help achieve a constant deposition rate.

2.0 COLLECTION AND ANALYSIS OF SAMPLES

An opaque leachate collection container of at least 200 litres volume should be used to collect leachate from the single drainage hole in the leachate collection tray. The container should be constructed with a replaceable polyethylene liner. For example, use a welded mild steel box with a drain pipe or a plastic children's wading pool which is lined and covered with black polyethylene. It is important to protect samples from degradation by ultraviolet light.

There are various ways that leachate can be collected from the tray. The collection containers must be compatible with the antisapstain chemical used in the test. For tetra and pentachlorophenols (CPs), 2-(thiocyanomethylthio)-benzothiazole (TCMTB), Copper-8-quinolinolate (Cu-8), Resin Acids, 3-iodo-2-propynyl butyl carbamate (IPBC) and borax/calcium carbonate active ingredients, use amber coloured borosilicate glass containers. These containers must be acetone rinsed and heat treated at 325°C for a minimum of twelve hours and sealed with heat treated aluminum foil liners. For didecyldimethyl ammonium Chloride (DDAC) active ingredients, opaque plastic containers should be used. Antisapstain formulations which are a mixture of chemicals will require the collection of samples for each active ingredient. For example a mixture of DDAC and IPBC will require one amber glass and one plastic container per sampling. For other antisapstain chemicals use the manufacturers recommended container types.

2.1 Collection and Testing of Wood Samples

Samples of wood should be collected from the control package and the treated lumber package, prior to exposure to rainfall, and analysed for resin acids and retention of antisapstain chemical. See Appendix II for the lumber sampling method developed Forintek Canada Corp.

After completion of benchmark rainfall cycles #1, 7, and 15 the control and the treated sets of lumber should be sampled and analysed for resin acids. The set of treated packages should be sampled and analysed for retention of the antisapstain chemical after each rainfall cycle.

2.2 Collection and Testing of Leachate Samples

The control package leachate should be sampled on day 1(15 mm) and analysed for the antisapstain chemical to confirm its absence. If the lumber is contaminated with antisapstain chemical it should be sampled on each day a resin acid sample is collected.

Leachate from the antisapstain treated lumber packages should be sampled on days 1(15 mm), 3(30 mm), 5(45 mm), 7(60 mm), 9(75 mm), 11(90 mm), 13(105 mm), and 15(120 mm) and analysed for the antisapstain chemical.

Samples of leachate should be collected from the untreated control and the treated set of packages on days 1(15 mm), 7(60 mm) and 15(120 mm) and analysed for resin acids.

Samples should be stored in a dark place at 4 Celsius or in coolers with crushed ice and analysed as soon as possible.

The analytical methods for resin acids and for leachate containing active ingredients including CP's, TCMTB, Cu-8, and IPBC are in Appendices III - VIII. (Note a suitable DDAC method is still under development). For leachate containing other active ingredients the manufacturers approved analytical method should be used. The detection limits for the active ingredients must be at or below the calculated 96 h LC50 for the most sensitive fish species. For more information on analytical procedures contact the:

Pacific Regional Office of Environment Canada
Conservation and Protection Laboratories
4195 Marine Drive, West Vancouver
British Columbia, Canada, V7V 1N8
(604)666-6767

2.3 96 Hour LC50 Static Fish Bioassays

A minimum of three samples for 96 Hour Static LC50 fish bioassays should be collected from the control and the treated lumber. Sampling should be conducted, on benchmark days 1(15 mm), 7(60 mm), and 15(120 mm). The bioassays should be conducted according to the method in Appendix IX.

3.0 DATA PRESENTATION

Total rainfall per leaching cycle should be plotted vs days of leaching as in Figure 3A. Natural rainfall should be included in the total for each day it occurs.

The chemical loading on the wood surface should be recorded in ug/cm^2 and plotted vs the days of leaching as in Figure 3B.

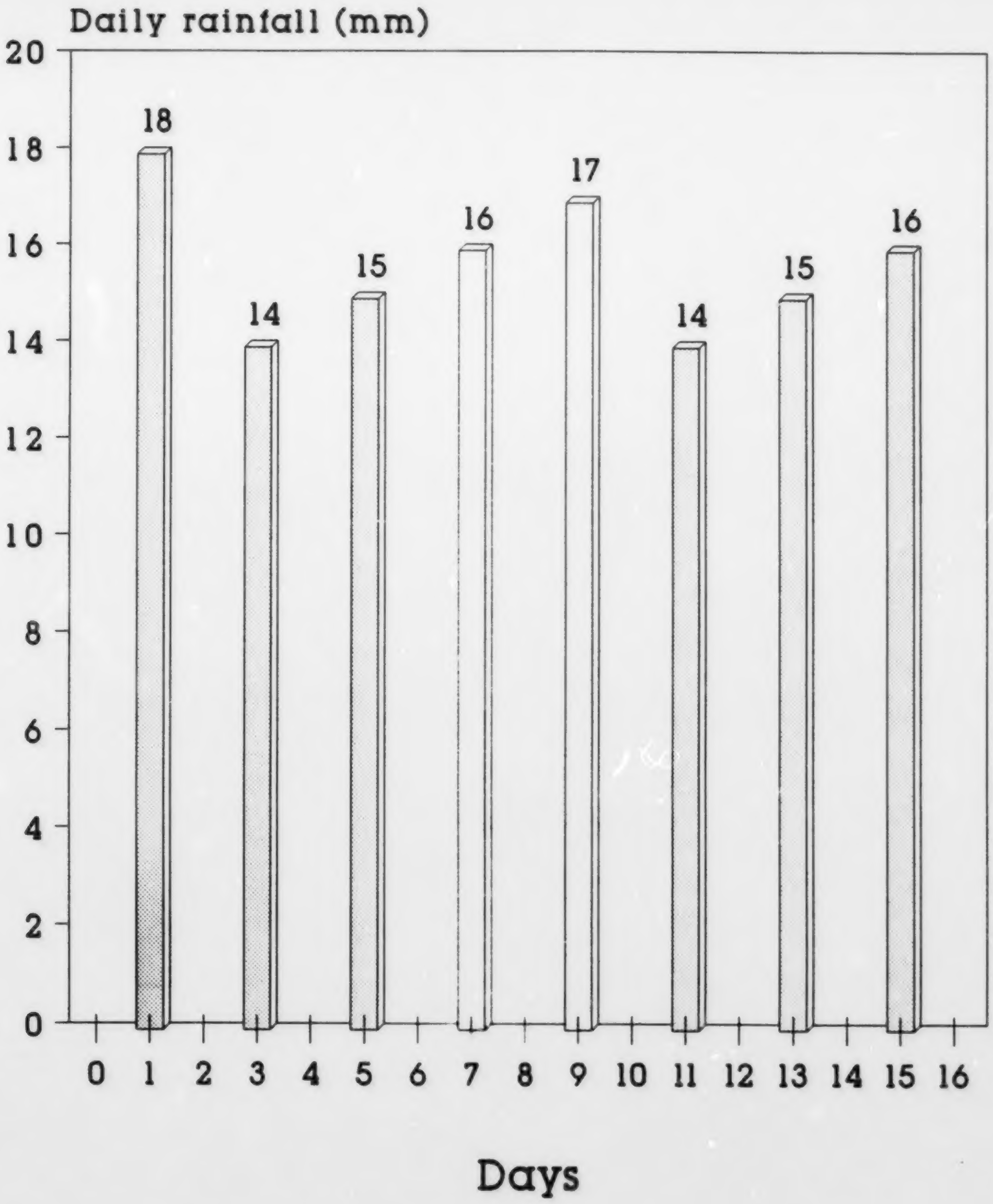
The concentration of antisapstain chemical in the leachate should be recorded and plotted vs the days of leaching as in Figure 3B.

The concentration of Resin Acids in the leachate and wood from the control package and the treated package should be plotted vs the days of leaching as in Figure 3C.

The maximum estimated volume of leachate based on total precipitation and the tray surface area and the actual volume of leachate collected should be recorded each day of leaching and reported as in Figure 3D. This is to determine loss of liquid due to retention by the wood during precipitation and evaporation from the wood after precipitation ends.

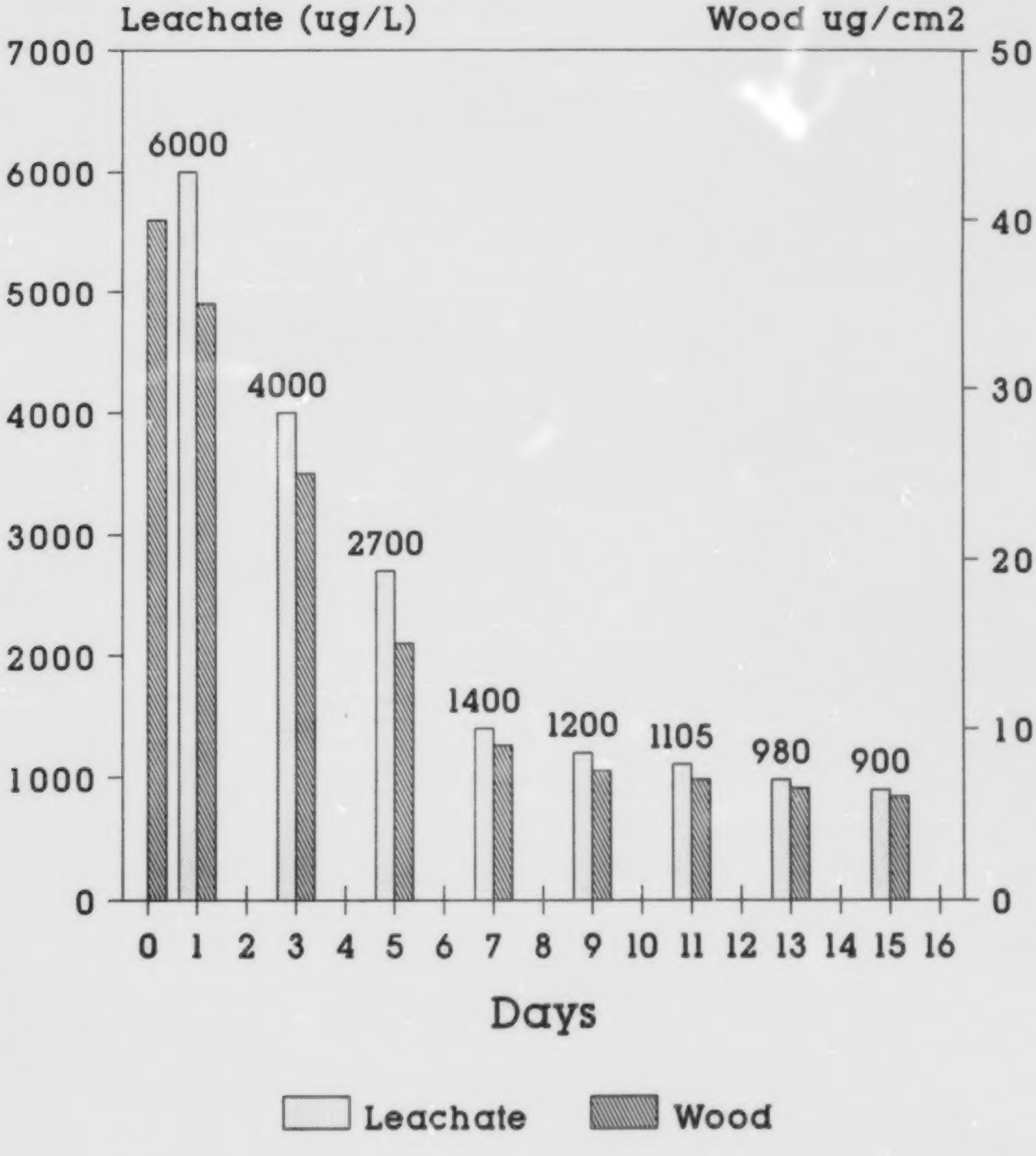
The quantity of antisapstain chemical lost due to leaching can be calculated by multiplying the concentration in leachate by the total volume collected each leaching cycle. Chemical lost per thousand board feet should be plotted as in Figure 3E.

Figure 3A: Daily Rainfall Accumulation



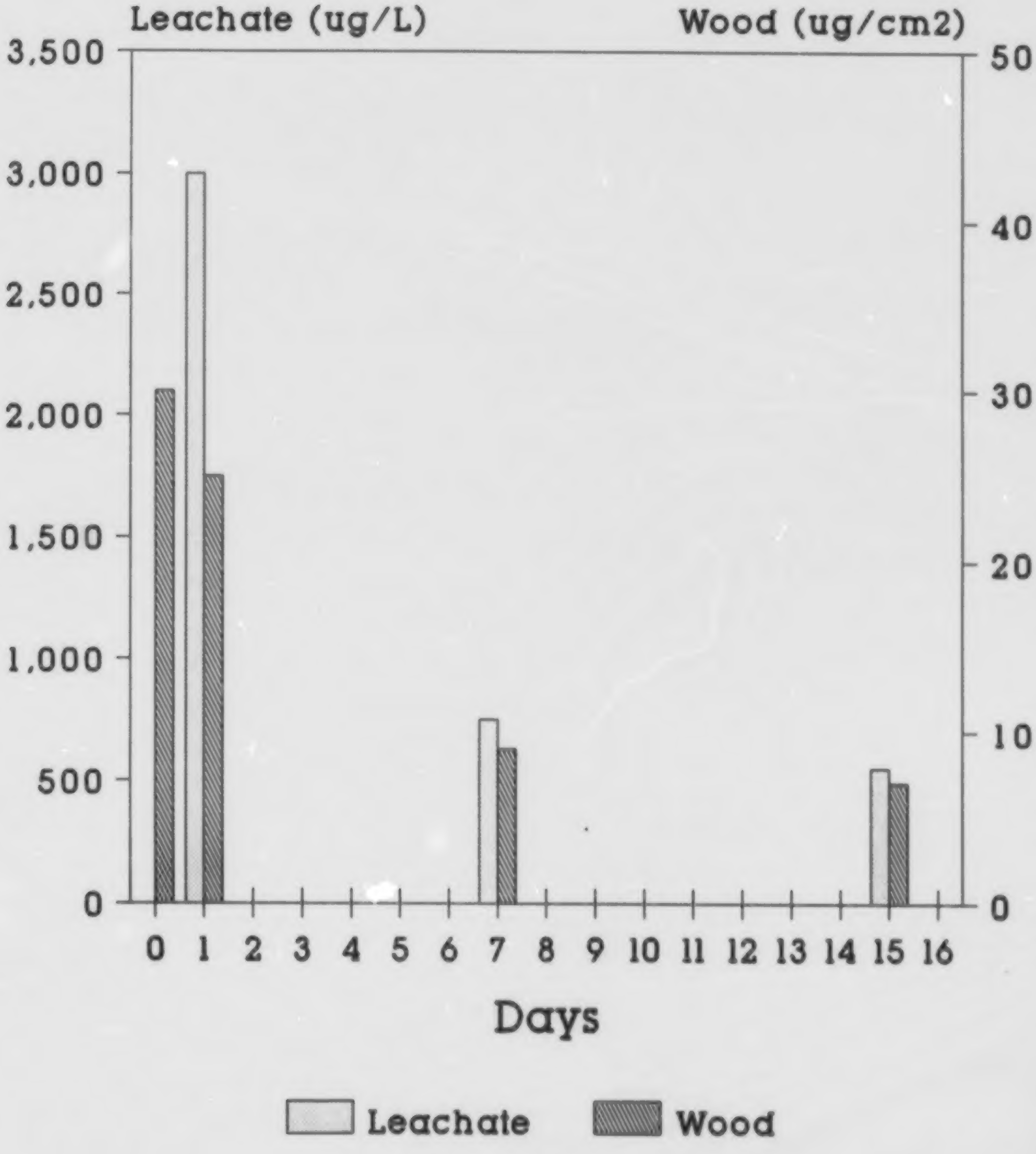
Simulated data as example only.

Figure 3B: Antisapstain Concentration vs Days with Precipitation



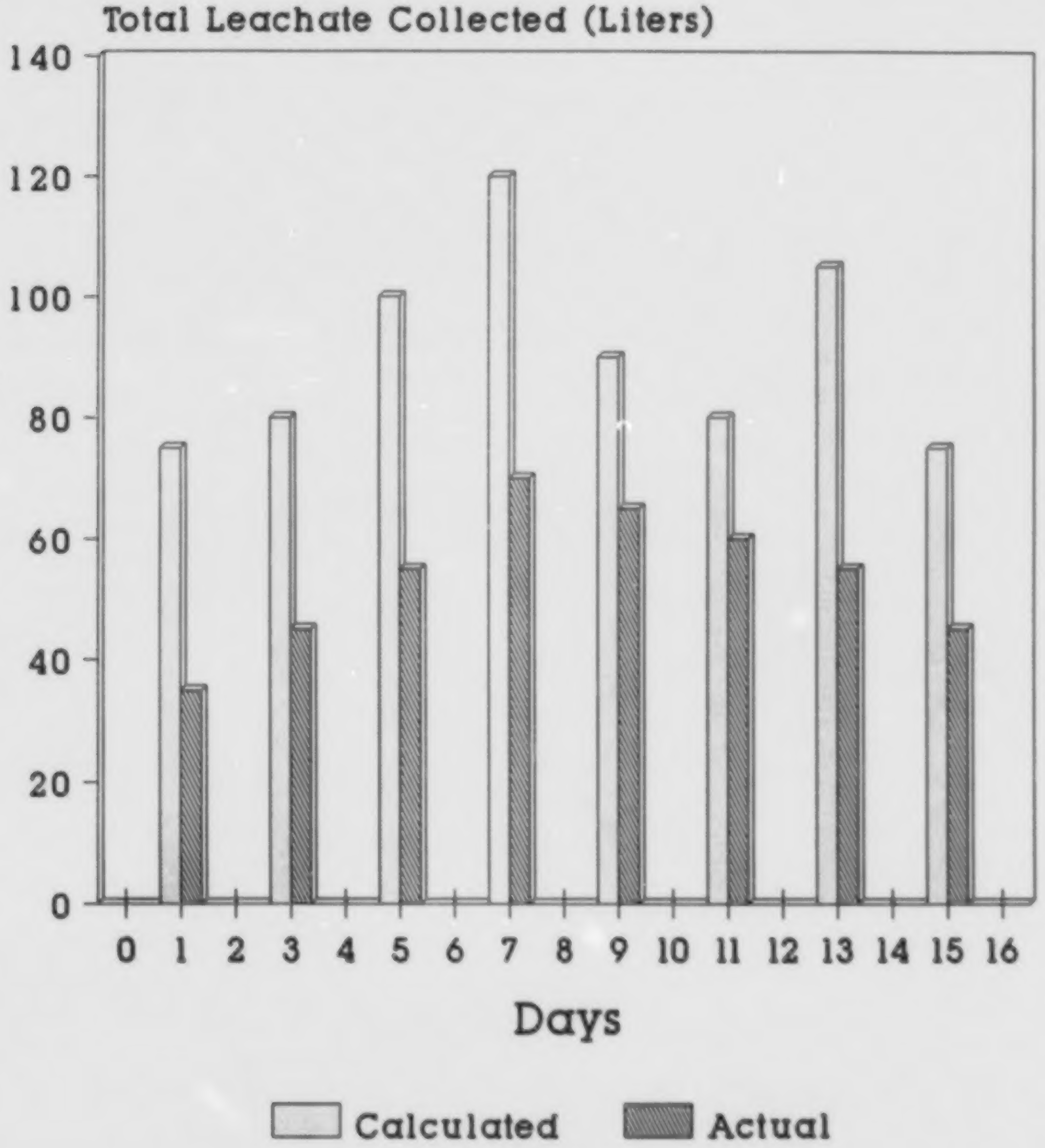
Simulated data as example only

Figure 3C: Resin Acids Concentration vs Days with Precipitation



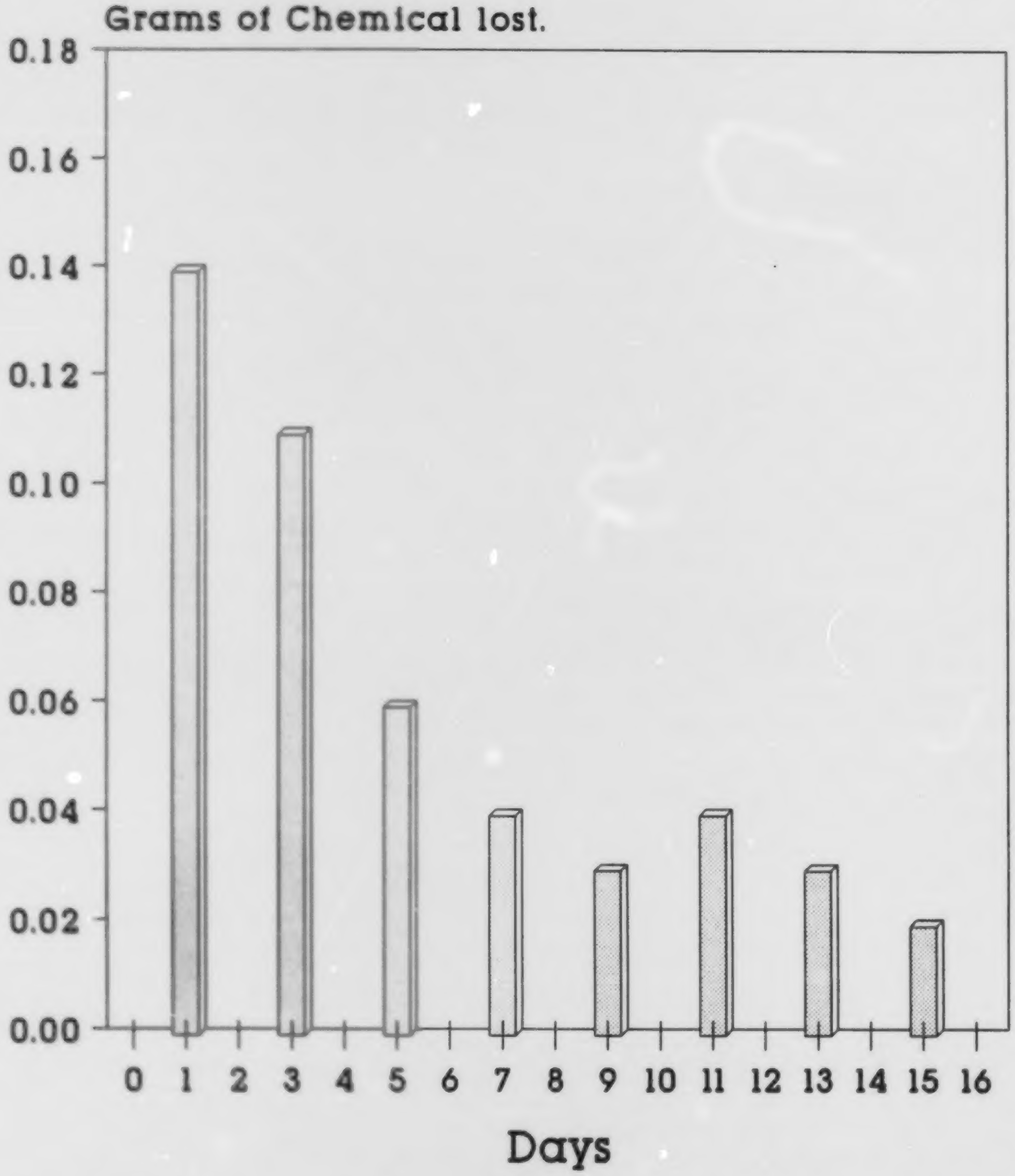
Simulated data as example only

Figure 3D: Total Leachate Volume Actual vs Calculated



Simulated data as example only.

Figure 3E: Total Antisapstain Chemical loss per leaching per 1000 Board Feet



Simulated data as example only.

4.0 ESTIMATION OF THE CONCENTRATION OF ANTISAPSTAIN CHEMICAL IN STORMWATER RUNOFF FROM A TREATED LUMBER STORAGE YARD

With the exception of some single product facilities most sawmills and export terminals produce and/or treat a wide range of lumber sizes and grades. Information provided by British Columbia lumber producers indicated that average storage time of treated lumber was in the range of two to four weeks. A typical production month will have at least 20 treating days and the average worst case rainfall month has at least 16 leaching cycles. As lumber is removed from the treating area it is sorted according to grade and length and moved to the appropriate area of the storage yard. This results in an essentially random distribution of freshly treated lumber around the storage yard which becomes mixed with lumber that has had post treatment storage times ranging up to one month.

The treating facility will constantly be producing lumber as new yard inventory and removing older lumber for shipment to market. The mix of production ages of treated lumber will remain relatively constant on the yard therefore any given storage yard will have 1/16 of the yard inventory leaching at the first cycle concentration, 1/16 at the second cycle rate etc. to 1/16 at the 16th cycle rate. Figure 3 shows that by the 8th leaching cycle the decrease in the concentration of antisapstain chemicals in the leachate can be approximated by a negative linear slope. To generate a reasonable estimate it is necessary to continue the leaching test until the linear portion of the leaching curve is reached. Tests conducted on four antisapstain chemicals have shown this to occur within 7 cycles or 105 mm of rainfall.

Samples of leachate dripping from lumber treated with antisapstain chemicals and yard runoff discharging into storm drains at working lumber mills and export terminals in British Columbia were collected in 1986, 1987, 1989, and 1990. The conditions at the various locations included all of the variables mentioned in section 1.2. A total of 16 sampling sequences were examined. As leachate dripped from the lumber and migrated to the storm outfall it became diluted with rainwater from open areas on the mill yard. The mean dilution ration was found to be 15 +/- 9 times.(1,3,6)

The average concentration of antisapstain measured in the 31 day leaching trial should be divided by the mean dilution ration of 15, and the range values of 6 and 23 to determine the "general industry wide" predicted yard runoff concentration. (A mill specific calculation would have to use the dilution ratio determined by the site specific storage yard size, lumber inventory and wood absorption ratios).

4.1 An Example of the Estimation of Antisapstain Concentrations in Lumber Leachate and Yard Runoff

An example of the concentration of a fictitious antisapstain in leachate is given in Figure 3B. The leachate concentration in the remaining 8 cycles can be extrapolated by calculating the slope of the linear portion of the experimental curve. Using the values of cycle 5 (C5) to Cycle 8 (C8) of Figure 3B, slope "S" is calculated where:

$$S = \frac{C8 - C5}{8 \text{ cycles} - 4 \text{ cycles}} = \frac{900 - 1200}{4 \text{ cycles}} = \frac{-300}{4 \text{ cycles}} = -75 \ \frac{\text{ppb}}{\text{cycle}}$$

$C_{leachate}$ for cycle 9 is calculated where:

$$C9 = C8 + S = 900 + (-75) = 825 \text{ ppb}$$

The values for all the cycles are:

From Figure 3B

cycle 1 = C1 = 6000
cycle 2 = C2 = 4000
cycle 3 = C3 = 2700
cycle 4 = C4 = 1400
cycle 5 = C5 = 1200
cycle 6 = C6 = 1005
cycle 7 = C7 = 980
cycle 8 = C8 = 900

Calculated Values

cycle 9 = C9 = 825
cycle 10 = C10 = 750
cycle 11 = C11 = 675
cycle 12 = C12 = 600
cycle 13 = C13 = 525
cycle 14 = C14 = 450
cycle 15 = C15 = 375
cycle 16 = C16 = 300

The average concentration of antisapstain chemical in the "leachate from the entire yard" is estimated by the summation of the concentrations for each cycle of the test packages divided by the total number of cycles.

$$C_{\text{Leachate}} = \frac{C1 + C2 + C3 + C4 + C5 + \ldots + C16}{16}$$

$$= \frac{22{,}685}{16}$$

$$= 1{,}417 \text{ ppb}$$

The average concentration of antisapstain chemical in the C_{runoff} from the entire yard is estimated by dividing C_{Leachate} by the average yard dilution factor of 15.

$$C_{\text{runoff}} = C_{\text{Leachate}}/15 = \frac{1{,}147}{15} \text{ ppb} = 95 \text{ ppb}$$

The range in concentration of antisapstain chemical in the "runoff" on an industry wide basis will depend largely on the yard dilution factors if the chemical application rate and minimum covered storage times are properly applied. The range for this example is:

$$\frac{1{,}147}{15 + 9} \text{ ppb} \leq C_{\text{runoff}} \leq \frac{1{,}147}{15 - 9} \text{ ppb}$$

$$59 \text{ ppb} \leq C_{\text{runoff}} \geq 236 \text{ ppb}$$

These estimates do not include factors such as deposition rates of chemicals to matrices such as storage yard surfaces and drainage system piping. Any deposition rates would have to be determined by experiment. The estimate does not include degradation by ultraviolet light which would have to be determined. The concentrations estimated here are "upper limits" of actual yard values.

If samples were collected at an actual operating facility concentrations outside this range would be due to abnormal operating conditions. Lower than 59 ppb would likely be due to a low lumber inventory in the yard, low chemical application rate or the addition of "dilution" water other than rainfall which impinges directly on the treated lumber storage yard. Higher than 236 ppb would likely be due to a high lumber inventory, higher chemical application rate or too short a drying time prior to exposure to rainfall.

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2) Krahn, P.K. "Computer Simulations of the Use of Diffusers to Reduce the Toxicity of Contaminated Storm Water Runoff from Treated Lumber Storage Yards". Environment Canada, Environmental Protection, Pacific and Yukon Region, Regional Data Report DR-88-02.

3) Krahn, P.K. "Leaching of 2-(thiocyanomethylthio)benzothiazole (TCMTB) from Surface Treated Lumber". Environment Canada, Environmental Protection, Pacific and Yukon Region, Regional Program Report 90-06 unpublished.

4) Fisheries Act, R.S.C., C. F-14, as amended by:
R.S.C., 1985, c.31 (1st supp.)
R.S.C., 1985, c.35 (1st supp.)
R.S.C., 1985, c.27 (2nd supp.)
Statutes of Canada 1988, c.2

5) Province of British Columbia, Waste Management Act, Section 35(2)(r) and (y) and (2.1), "Antisapstain Chemical Waste Control Regulation", 1990.

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10) Martin, H.V. "Mill Antistain Treatment Using TCMTB", Project BD-301, Report No. 2, "The Leaching of Busan 1030 from Dip Treated Lumber", MacMillan Bloedel Research, December 8, 1986. Used by permission.

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APPENDIX I

Province of British Columbia, Waste Management Act, Section 35(2)(r) and (y) and (2.1), "Antisapstain Chemical Waste Control Regulation", 1990

Note: This regulation is subject to review and amendment from time to time. Current versions of the regulation may be obtained from:

Government of British Columbia
Ministry of Environment and Parks
Waste Management Branch
810 Blanshard Street
Victoria, British Columbia
Canada, V8V 1X5

(604)387-9993

NCE OF BRITISH COLUMBIA

ORDER OF THE LIEUTENANT GOVERNOR IN COUNCIL

der in Council No. 1289, Approved and Ordered AUG 30 1990

Lieutenant Governor

Executive Council Chambers, Victoria AUG 29 1990

On the recommendation of the undersigned, the Lieutenant Governor, by and with the advice and consent of the Executive Council, orders that

(a) the Antisapstain Chemical Waste Control Regulation, B.C. Reg. 258/89, is repealed, and

(b) the regulation in the attached schedule is made.

DEPOSITED
AUG 31 1990
B.C. REG. 300/90

Minister of Environment

Presiding Member of the Executive Council

(This part is for administrative purposes only and is not part of the Order.)

uthority under which Order is made:

Act and section: Waste Management Act, section 35 (2) (r) and (y) and (2.1)

SCHEDULE

ANTISAPSTAIN CHEMICAL WASTE CONTROL REGULATION

Interpretation

1. (1) In this regulation

"antisapstain chemical" means chlorophenol, 2-(thiocyanomethylthio) benzothiazole (TCMTB), copper-8-quinolinolate (Cu-8), 3-iodo-2-propynyl butyl carbamate (IPBC) and didecyldimethyl ammonium chloride (DDAC);

"chlorophenol" means the sum of chlorophenols plus chlorophenates as reported by a laboratory testing method approved by the director;

"curb" means a raised edge or curbing which would confine all liquids dropping within the enclosure formed by it and the surface on which it sits;

"effluent" includes precipitation runoff;

"fixed roof" means a roof which is designed and constructed to prevent the entry of precipitation and to be a maintenance free structure for a period of not less than 10 years, but does not include a structure which utilizes tarpaulins or similar materials;

"impervious" means not allowing entrance or passage of antisapstain chemicals;

"isolated" means set apart from other operations in an enclosure away from any high traffic areas that can, under normal circumstances, prevent the entry of unauthorized persons.

(2) For the purposes of this regulation effluent is toxic if the effluent at 100% concentration kills 50% or more of rainbow trout within 96 hours when tested in accordance with procedures approved by the director.

Prohibition

2. A person shall not introduce into the environment

(a) an effluent that contains antisapstain chemicals from

(i) sawmills, lumber export terminals and other facilities that, for the purpose of preventing sapstain growth, apply antisapstain chemicals or have during the 12 months immediately preceding the introduction of the effluent applied antisapstain chemicals, or

(ii) storage areas for lumber treated with antisapstain chemicals, not including storage yards at facilities which only store lumber for the purpose of retail sale directly to the public,

(b) emissions to the air from antisapstain chemical spray booth vents, or

(c) emissions to the air consisting of combustion products of antisapstain chemicals and their byproducts

unless the person

(d) meets the requirements under sections 4 to 12 where the person used or uses chlorophenol, TCMTB, DDAC, IPBC or Cu-8, and

(e) complies with a monitoring program, if any, established by the manager that allows the manager to determine

(i) the concentration of antisapstain chemical in effluents,

(ii) the toxicity of the effluents, or

(iii) the rate of antisapstain chemical emissions to the air from antisapstain chemical spray booths.

Exemption

3. A person to whom section 2 applies is exempt from section 3 of the *Waste Management Act* in respect of the introduction into the environment of an effluent or emission referred to in section 2 (a) to (c) if the person meets the requirements of section 2 (d) and (e).

Storm sewer design

4. (1) A storm sewer serving a storage area referred to in section 2 (a) (ii) or any antisapstain treatment area of any facility referred to in section 2 (a) shall receive only precipitation and runoff from the storage area, treatment area or both, including roof drainage from structures which shelter the storage areas and treatment areas, and shall convey it to a sampling point downstream of the storage area and treatment area.

(2) Notwithstanding subsection (1), the storm sewer may receive and convey additional liquids downstream of the sampling point.

Quality of effluent including stormwater

5. (1) Up to and including August 31, 1990, the concentration of an individual antisapstain chemical in effluent shall not exceed 6 micrograms per litre (µg/ℓ) chlorophenol, 200 µg/ℓ TCMTB or 200 µg/ℓ Cu-8.

(2) On and after September 1, 1990 until September 1, 1991, the concentration of an individual antisapstain chemical in effluent shall

not exceed 6 µg/ℓ chlorophenol, 15 µg/ℓ TCMTB, 15 µg/ℓ Cu-8, 120 µg/ℓ IPBC or 700 µg/ℓ DDAC.

(3) On and after September 1, 1991 the concentration of an individual antisapstain chemical in effluent shall not exceed 6 µg/ℓ chlorophenol, 6 µg/ℓ TCMTB, 15 µg/ℓ Cu-8, 120 µg/ℓ IPBC or 700 µg/ℓ DDAC.

(4) On and after September 1, 1990, the effluents from operations described in section 2 (a) shall not be toxic.

(5) Sampling to determine a concentration of any individual antisapstain chemical in effluent under subsection (1), (2) or (3) shall occur at the sampling point referred to in section 4.

(6) Sampling referred to in subsection (4) and the analysis of the samples shall be conducted using sampling and analytical methods approved by the director.

Design of antisapstain chemical storage and mix facilities

6. (1) An antisapstain concentrated chemical storage system, concentrate feed system, mix system and working solution storage tanks

(a) shall be located in an isolated area within a building, and

(b) shall have doors that provide access to the area and that shall be kept locked except when a person is present for work related purposes.

(2) A spill containment system shall be maintained for the isolated area under subsection (1) and the spill system shall be impervious and of sufficient size to contain at least 120% of the volume of the largest tank in the isolated area.

Design of antisapstain chemical dip tank systems

7. (1) An antisapstain chemical dip tank shall

(a) be impervious,

(b) if it is an above ground tank, be located in an impervious containment area which has

(i) a sloped floor, and

(ii) a curb that is of sufficient height to enable the containment area to hold at least 120% of the largest chemical dip tank volume, and that prevents entry of surface runoff,

(c) if it is not an above ground tank, be a double wall containment tank constructed with

- (i) both walls impervious, and
- (ii) a means for determining if there is leakage of the chemicals into the area between the walls,

(d) have an associated impervious primary drip area that provides 30 minutes minimum dripping time for treated wood immediately following the treatment and that prevents entry to the dip tank of surface runoff and precipitation,

(e) have a system for recycling all drippings from the containment area referred to in paragraphs (b) and (c) and from the impervious drip area referred to in paragraph (d) back to the dip tank, and

(f) be constructed so as to prevent a motorized vehicle of a type that can reasonably be expected to operate near the dip tank from driving in or through the dip tank.

(2) The containment area referred to in subsection (1) (b), the tank referred to in subsection (1) (c) and the primary drip area referred to in subsection (1) (d) shall be provided with a fixed roof and with guard rails or equivalent to provide vehicle impact protection.

(3) Up to and including August 31, 1990, the requirements contained in subsections (1) (c) and (f) do not apply to antisapstain chemical dip tanks constructed before August 25, 1989.

Design of antisapstain chemical spray booth systems

8. (1) An antisapstain chemical spray booth system shall have

(a) a collection tray that is impervious and that facilitates recycling for re-use of all oversprayed antisapstain chemicals,

(b) under both the spray box and collection tray, a containment area that

- (i) consists of a floor and curb,
- (ii) is impervious, and
- (iii) has the capacity to contain all drippings for subsequent recycling or for disposal in accordance with the provisions of the Special Waste Regulation (B.C. Reg. 63/88), and

(c) a primary drip area that

- (i) provides 30 minutes minimum drying time for treated wood immediately following treatment,
- (ii) has a fixed roof, and
- (iii) has a system for recycling all drippings for re-use.

(2) Emissions to the air from antisapstain chemical application spray booths

- (a) shall be treated by a demister or an alternative approved by the manager and then
  - (i) shall be vented directly to the atmosphere by a stack, the top of which shall be at least 10 metres above the ground and 5 metres above the highest point of the roof of the building which houses the antisapstain chemical spray booth, or
  - (ii) all or part of the treated emission shall be recirculated back to the spray booth, and
- (b) shall not be combined with any pneumatic stream that conveys chips, sawdust, planer shavings, hog fuel or any other wood residue.

Demister purge

9. Where a manager has issued a directive to minimize the quantity of antisapstain chemicals introduced to the environment by effluent purged from a demister referred to in section 8 (2) (a), the effluent shall be handled, treated and disposed of in accordance with the directive.

Antisapstain chemical spray booth atmospheric emission quality

10. The rate of emissions to the air from an antisapstain chemical spray booth stack referred to in section 8 (2) (a) shall not exceed

- (a) 7.0 mg per second chlorophenol,
- (b) 7.0 mg per second TCMTB,
- (c) 7.0 mg per second Cu-8,
- (d) 7.0 mg per second DDAC, or
- (e) 7.0 mg per second IPBC

as determined by sampling and analytical methods approved by the director.

Management of sludges and wood residue

11. (1) Sludges that qualify as special waste and that have originated from antisapstain chemical dip tanks and spray system filtering or screening processes shall not be incinerated, buried or otherwise disposed of or introduced into the environment unless all provisions of the Special Waste Regulation are satisfied.

(2) Trim ends, broken lumber, chips, sawdust, planer shavings and other wood residue resulting from processing and handling of wood following its treatment with antisapstain chemicals

(a) shall not be provided to a person for use as mulch or for burning in a residential fireplace, stove or other burning device other than an incinerator permitted under paragraph (b),

(b) shall be incinerated only in a hog fuel burning incinerator, or other incineration device, that is designed

(i) to burn the wood residue at a minimum combustion temperature of 900° C, and

(ii) for gases in the combustion zone to have a minimum residence time of one second, and

(c) if the antisapstain chemical is a chlorophenol, shall not be provided to a person for the purpose of supplying digesters in pulp manufacturing processes.

Design of treated wood storage areas

12. Lumber treated with antisapstain chemicals and not otherwise protected from contact with precipitation shall be stored in an area that has a floor and a curb that are impervious to the treatment chemicals or that are constructed of asphalt that is maintained in good condition.

APPENDIX II

Forintek method for sampling the surface of wood treated with antisapstain chemicals

Forintek Lumber Sampling Protocol for Antisapstain Chemical Retention

Rationale

The control of moulds and staining fungi by chemicals is based on a knowledge of the efficacy of the chemical. By efficacy testing we determine how much of the chemical, in terms of active ingredient per surface area, is required to protect the wood. Although sawmills have variable methods of chemical application and treatment solution concentrations it is the amount of chemical on the wood surface which is the important factor. Application lower than the recommended rate will result in problems in the marketplace while application above this rate is a waste of chemical. In chemical leaching tests it is important that the surface retention of the wood is determined.

Method

In the case of antisapstain products which are known to diffuse into the wood, the samples should be taken as soon after treatment as possible so that the chemical has not penetrated beyond the sampling depth.

Replication

Sufficient samples to enable determination of an average treatment level should be analysed. The more uneven the wood is (in texture, size and treatment) the more samples will have to be taken to get a representative average. Generally about 20-samples from a package will give a reasonable estimate of the average treatment level. However for samples being treated in a cross-chain spray system which is not applying a very even treatment, considerably more samples may be required.

Where to sample

Because spray systems do not necessarily spray evenly on all faces and sides of the lumber each face of the wood should be sampled. For longitudinal spray systems and diptanks sample the top, bottom and sides in the centre part of random pieces from a package. Samples taken each from a different board will give the best "average" retention. Split the 20 samples between the top, bottom and sides in a manner roughly proportional to the size of the pieces. For example, for Japanese squares you would do five of each side, for 2" x 10" lumber you could do 4 sides, 8 tops and 8 bottoms.

Special Tools

Samples are taken with a 6.5 cm^2 special punch obtainable from the manufacturer, Orion Engineering Ltd., 700 Copping St. North Vancouver, British Columbia, Canada, V7N 3G6, Telephone (604)987-7617. The punch has been precision engineered to accurately sample this size of area. Do not sharpen in such a way as to change the area of the punch.

Obtaining a Sample

The wood to be sampled should be on a solid base. When choosing a position on the board to sample the wood remember that it is difficult where the grain is uneven (such as around knots) and the easiest place to sample is on straight-grained wood. Place the punch over the spot to be sampled and strike with a small (four-pound is ideal) sledge hammer. As with any chisel you will require safety glasses when striking the punch. Two or three sharp blows should drive the punch into the wood to the point where you can lever out the sample. With a little practice a sample can be removed in one piece. So long as the entire surface is removed it does not matter if the sample is in one piece or several. The sample removed need be no thicker than 3 mm.

Transporting Samples.

The samples should be allowed to surface dry for a few minutes before being placed in small plactic bags, or, preferably, in extraction vials. Labelling should be done immediately and additional information reported in a notebook. The samples should be sent promptly to the analytical laboratory. If delays occur the samples must be refrigerated.

Analysis

The samples should be analysed in a laboratory which has a proven analytical method for analysing the chemical on a wood matrix.

Reporting of results

Report the individual analyses, the average, and the range of retentions.

APPENDIX III

Environment Canada method for chlorophenols analysis

Environment Canada
Conservation and Protection

Laboratories
Pacific & Yukon Region

CHLORINATED PHENOLS

V2.2 April, 1990

CP

Gas Chromatographic - Diazomethane derivative

SCOPE AND APPLICATION

This method is applicable to the qualitative and quantitative electron capture gas chromatographic determination of Pentachlorophenol, 2,3,4,5-Tetrachlorophenol and 2,3,4,6/2,3,5,6-Tetrachlorophenol in effluents, sediments and biota. PCB's may also be determined using this procedure by an appropriate split of the extract.

SAMPLE HANDLING AND PRESERVATION

Note: All glassware, aluminum foil and sampling equipment that comes in contact with the sample must be acetone rinsed and heat-treated at 325 C for a minimum of 12 hr.

1. Effluent samples should be collected in an all glass system and stored at about 4 C. If teflon-lined screw closures are not available for the sample containers then heat-treated aluminum foil must be used as a liner. At least 1 litre of effluent should be collected.
2. Sediment samples should be collected in an all glass system and frozen as soon as possible. If teflon-lined screw closures are not available for the sample containers then heat-treated aluminum foil must be used as a liner. The sample weight collected should be at least 100 grams.
3. Small biota samples should be wrapped in heat-treated aluminum foil, placed in a heat-treated glass bottle and frozen as soon as possible. Larger biota samples should be dissected and treated as above prior to submission. The sample weight collected should be at least 25 grams. If teflon-lined screw closures are not available for the sample containers then heat-treated aluminum foil must be used as a liner.

SUMMARY OF METHOD

Effluent samples are acidified with dilute sulfuric acid and the chlorinated phenols extracted with diethyl ether. After concentration the extract is methylated using ethereal diazomethane, then treated with Florisil, activated copper, concentrated sulfuric acid and mercury to remove interferences then analyzed using packed column electron capture gas chromatography.

Sediment and homogenized biota samples are acetone extracted using a wrist-action shaker, partitioned with 2% NaCl, methylated, then cleaned up and analyzed in the manner outlined for effluent samples.

contd. ...

METHOD PERFORMANCE

1. The minimum detectable concentration of pentachlorophenol in effluents and sediment/biota samples is 0.002 ug/l and 0.0001 ug/g respectively. The tetrachlorophenols similarly provide 0.005 ug/l and 0.0002 ug/g as detection limits. These results were obtained using a packed column gas chromatograph with electron capture detector.
2. The electron capture detector has been show to be linear over the range of 0.001-0.5 ng injected standard for pentachlorophenol and 2,3,4,6-tetrachlorophenol with a correlation coefficient of 0.999998 over 6 data points.
3. Quality Control Data

Sample Type	Pentachlorophenol		Tetrachlorophenol	
	accepted value	found value	accepted value	found value
Commercial Phenol Mix - ug/ml (Foxboro RCS-170P)	0.066	0.066	-	-
Water - ug/l (FICP Interlab, 870452)	10.3	7.0	11.1	10
	5.2	5.0	11.1	10
	10.3	6.9	22.0	19
Fish oil - ug/g (CCIW Interlab)	2.12	2.49	2.00	2.39
	0.53	0.70	0.50	0.60
Fish flesh - ng/g (CCIW Interlab)	52.7	53	27.5	46
	52.7	55	27.5	45

4. In-House Recovery Data of Chlorinated Phenols from 750 ml of Fortified Wastewater.

Chlorinated Phenol	Fortification Level (ug/l)	Average Recovery (%)	Coefficient of variation (%)
2,3,5,6-Tetrachlorophenol	1.0	91	3.6
2,3,4,5-Tetrachlorophenol	1.0	90	4.3
Pentachlorophenol	1.0	96	2.4

contd. ...

5. In-House Reproducibility Data for Samples Analyzed

Chlorinated Phenol /Sample Type	N	T	AVG % Standard Deviation
2,3,4,6-Tetrachlorophenol			
/effluent	88	21 mos	6.4
/sediment	134	21 mos	28.0
/biota	38	21 mos	48.9
/(combined)	(260)	21 mos	(23.7)
Pentachlorophenol			
/effluent	88	21 mos	5.2
/sediment	134	21 mos	30.7
/biota	38	21 mos	41.7
/(combined)	(260)	21 mos	(23.7)

N = number of duplicate analyses

T = period of time

Note: as the duplicate samples were not split from a previously homogenized total sample it is felt that the relatively large % Standard Deviation in the sediments and biota samples are mainly due to heterogeneity within the sample.

INTERFERENCES

1. Solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of the chromatograms. All of these materials must be demonstrated to be free of interferences under the conditions of analysis.
2. Organochlorine pesticides, certain organophosphorous pesticides, other halogenated compounds, phthalate esters, and elemental sulphur constitute potential interferences in the determination of CP´s. Most of these are eliminated by the described cleanup method. Common formulations of CP wood preservatives have high percentages of tetra- and penta- chlorophenols. If only a pentachlorophenol peak, without a corresponding tetrachlorophenol peak, is present be aware that a few organochlorine pesticides elute in this area.
3. Hydrocarbons may create analytical problems, such as quenching of the EC detector, especially when their concentration considerably exceeds that of the CP´s. Hydrocarbons, which co-elute from the 2% Florisil column with the CP´s, are usually destroyed by the sulfuric acid clean-up step detailed in this procedure.
4. Difficulties may be encountered in the analysis of pulp mill waste due to the interference of chlorinated degradation products of lignin.

contd. ...

INTERFERENCES (ctd.)

5. The 2,3,4,6-tetrachlorophenol isomer is not chromatographically resolved from 2,3,5,6-tetrachlorophenol with the recommended gas chromatographic columns. While these isomer co-elute, their ECD response factors are quite different. In wood preservatives, the common source of CP´s, 2,3,4,6-tetrachlorophenol has been identified by GC/MS as the isomer present, thus results should be quantitated on the basis of this isomer.
6. If emulsions form during the extraction, add a few drops of a saturated sodium sulfate solution and gently agitate. If the emulsion persists then transfer the extract to a glass centrifuge tube and centrifuge for 30 minutes at 2500 rpm.

SAFETY

1. Chlorinated phenols are toxic chemicals. Exercise great care in the handling of samples and their extracts.
2. The following is extracted from Aldrich Technical Information Bulletin #AL-113 concerning the generation of diazomethane:

CAUTION: USE ONLY THE GLASSWARE SUPPLIED WITH THE DIAZALD KIT. THE USE OF STANDARD GROUND GLASS JOINTS WILL CREATE AN EXTREME EXPLOSION HAZARD. DIAZOMETHANE MUST BE GENERATED ONLY IN AN EFFICIENT FUME HOOD AND BEHIND A SAFETY SHIELD. DIAZOMETHANE IS EXTREMELY TOXIC.

APPARATUS

All glassware must be washed with heavy-duty soap, rinsed with distilled water, rinsed with acetone and then heat-treated at 325 C for at least 12 hours prior to use. The glassware must be stored in an area protected from chemical contamination.

1. Graduated cylinders, 500-ml.
2. Boiling flasks, 250-ml, 500-ml.
3. Separatory funnels, 250-ml, 500-ml.
4. Vials, 7-ml, with foil or teflon cap liners.
5. Volumetric flasks, 5-ml, 10-ml.
6. Buchner funnels, 6.5 cm.
7. Vacuum adapters, 24/40.
8. Brinkman Polytron homogenizer.
9. Homogenizer tubes, 5-cm X 5-cm X 12-cm.
10. Screw top centrifuge tubes, 100-ml, with teflon cap liners.
11. Rotary evaporator.
12. Wrist-action shaker.

contd. ...

APPARATUS (ctd.)

13. Ultrasonic cleaner.
14. Vortex mixer.
15. Glass columns, 26 cm x 2.0 cm, with sintered glass frits and teflon stopcocks.
14. *Diazald/Diazomethane Generator Kit, Aldrich #Z10,025-0.*
15. Packed Column Gas chromatograph, HP 5830A with dual EC detectors, on-column injectors and autosampler.
16. Gas chromatographic columns - 180 cm x 2 mm ID.
    (a) Primary - 4% OV-101/6% OV-210 on 80/100 Chromosorb W.
    (b) Secondary - 3% OV-1 on on 80/100 Chromosorb W.

REAGENTS

Note: All reagents must be shown to contain no interfering substances by running the appropriate blanks.

1. Hexane, pesticide grade.
2. Acetone, pesticide grade.
3. Diethyl ether, pesticide grade.
4. Ethanol, 95%, pesticide grade.
5. Methanol, pesticide grade.
6. Iso-octane, pesticide grade.
7. Sulfuric acid, conc., reagent grade.
8. Sulfuric acid, 2N.
    - carefully add 5.6 ml conc. sulfuric acid to 100 ml DI water.
9. Hexane extracted tap water.
    - extract 1500 ml of tap water with 200 ml of hexane in a 2-l sep. funnel.
    - let phases separate for 15 minutes then discard hexane fraction.
    - repeat extraction twice more.
10. Sodium sulfate, anhydrous, reagent grade.
    - heat-treat for 12 hr at 325 C.
    - wash with pesticide grade solvent of choice prior to use.
11. Sodium chloride, reagent grade.
    - heat-treat for 12 hr at 325 C.
    - wash with pesticide grade solvent of choice prior to use.
12. Sodium chloride solution, 2%.
    - make up using hexane extracted tap water.
13. Sodium hydroxide, reagent grade.
14. Potassium hydroxide, reagent grade.
15. Florisil, 100/200 mesh.
    - heat at 675 C for a minimum of 4 hr.
    - store at 130 C until used.
16. Florisil, 2%.
    - use 100 g of heat-treated Florisil cooled to R.T. in a dessicator in a 500-ml bottle with teflon-lined screw closure.

contd. ...

REAGENTS (ctd.)

16. Florisil, 2%. (ctd.)
    - quickly add exactly 2 ml of hexane extracted tap water.
    - tightly close the bottle and mix this by rolling for at least 2 hours.
    - when using keep the bottle tightly capped as much as possible.
17. Copper, activated.
    - rinse the copper powder with 2N HCL for 30 seconds then filter and rinse quickly with DI water until the wash is of neutral pH.
    - wash with acetone 4 times and air dry.
    - wash with hexane 3 times.
    - store under hexane in a closed bottle. It should be shiny for optimum effectiveness.
18. Mercury.
19. Diazald (N-methyl-N-nitroso-p-toluenesulfonamide), Aldrich #D2,800-0.
20. Diazomethane, ethereal alcoholic solution.
    - assemble the glassware kit according to the instructions contained in Aldrich Technical Information Bulletin #AL-131. Diazomethane is generated from the Diazald reagent.
    - a 250-ml distilling flask is fitted with a dropping funnel and an efficient condenser set for downwards distillation. The condenser is fitted to 2 receiving flasks in series, the second of which contains 30 ml of diethyl ether. The inlet tube of the second receiver dips below the surface of the ether and both receivers are cooled in a water-ice bath. Heat is supplied by a water bath set to 65 C. The heated portion of the glassware is covered with aluminum foil to promote faster distillation.
    - using the 250-ml distilling flask dissolve 5 g KOH in 8 ml DI water.
    - add 25 ml 95% ethanol.
    - add 21.4 g (0.1 mole) Diazald to 200 ml diethyl ether, dissolve and add to the dropping funnel.
    - over a period of 25 minutes add this to the alkali solution. The rate of addition should equal the rate of distillation.
    - when the dropping funnel is empty slowly add another 40 ml of diethyl ether and continue distillation until the distillate is colourless.
    - the combined ethereal distillates contain about 3 g of diazomethane.
    - store diazomethane over ether-washed NaOH in a glass bottle with a teflon top (NOT A GROUND GLASS TOP).
    - store the prepared diazomethane in the freezer compartment of an EXPLOSION PROOF refrigerator.
20. Pentachlorophenol, primary standard.
21. 2,3,5,6-Tetrachlorophenol, primary standard.
22. 2,3,4,5-Tetrachlorophenol, primary standard.
23. 2,3,4,6-Tetrachlorophenol, primary standard.
24. 2,3,6-Trichlorophenol, primary standard.
25. 2,4,6-Trichlorophenol, primary standard.

contd. ...

PROCEDURE - GENERAL

1. Full method blanks are to be run at least on a daily basis.
2. At least one sample in five (20%) should be run as a duplicate.
3. CP standards should be injected frequently as a check on the stability of the GC. At a minimum, standards are required at the beginning and end of an autosampler run.

PROCEDURE - EFFLUENT SAMPLES

1. Place 300 ml of the shaken sample in a 500-ml separatory funnel. If the sample is suspected to contain high CP levels then a smaller volume should used and diluted to about 300 ml with hexane-extracted tap water.
2. Carefully acidify the sample to pH 2 with 2N H_2SO_4.
3. Add 60 ml of diethyl ether to the graduated cylinder used to measure the sample, rinse and then transfer to the separatory funnel.
4. Shake vigorously for 2 minutes.
5. Drain the ether fraction into a 250-ml rotary evaporator flask.
6. Repeat steps 3, 4 and 5 twice more, combine and dry the extracts over anhydrous Na_2SO_4 for 15 minutes then transfer with washing to a clean 250-ml rotary evaporation flask.
7. Add 1 ml iso-octane as a keeper.
8. Concentrate to about 2 ml on a rotary evaporator. Caution - do not evaporate to dryness. Water bath temperature must not exceed 35 C. Make the sample up to exactly 5 ml using diethyl ether then split and workup as required if PCB's are to be run.
9. Proceed to the "COMMON TO ALL EXTRACTS" section.

PROCEDURE - SEDIMENTS AND BIOTA

1. For sediment samples weigh 15 grams of air-dried sample into a 100-ml centrifuge tube with teflon-lined screw closure and add 50 ml of acetone. Proceed to Step 3. Results are to be reported as "Dry Weight" (i.e. based on a sample air-dried at room temperature to constant weight). PCB's can also be determined from this extract.
2. For biota samples weigh 15 grams of frozen sample into a square homogenization tube, add 50 ml of acetone then homogenize the sample for 3 minutes at a Polytron speed setting of "4" maximum (to avoid mechanical losses). Quantitatively transfer the homogenized sample to a 100-ml centrifuge tube with teflon-lined screw closure and proceed to Step 3. Results are to be reported as "Wet Weight" (i.e. based on an "as received" weight). PCB's can also be determined from this extract.
3. Using a wrist-action shaker agitate the sample for 2 hours.
4. Filter the sample through a Buchner funnel, with 24/40 vacuum filtration adaptor and GF/C filter paper, into a 500-ml round bottom flask.
5. Rinse the centrifuge tube with 10 ml of acetone and filter, repeat twice more and combine the extracts.

contd. ...

PROCEDURE - SEDIMENTS AND BIOTA (ctd.)

6. Transfer the extract to a 250-ml separatory funnel and add 60 ml of 2% NaCl solution.
7. Extract with 50 ml hexane, repeat twice more and combine the extracts.
8. Add 1 ml iso-octane as a keeper.
9. Add 5 g anhydrous sodium sulfate, mix, let sit for 15 minutes to dry the extract, then transfer with hexane washing into a clean 500-ml rotary evaporator flask.
10. Concentrate to about 2 ml on a rotary evaporator. Caution - do not evaporate to dryness. Water bath temperature must not exceed 35 C. Make the sample up to exactly 5 ml using hexane and split as required if PCB's are to be run.
11. Proceed to the "COMMON TO ALL EXTRACTS" section.

PROCEDURE - COMMON TO ALL EXTRACTS

1. Transfer a 2 ml aliquot of the sample to a 7-ml vial with foil lined screw closure. If high CP levels are suspected then use an appropriate dilution.
2. In a fumehood, evaporate to very near dryness under nitrogen.
3. Add 0.25 ml of methanol and mix.
4. IN A FUME HOOD AND WITH EXTREME CAUTION, add 2 ml of diazomethane, cap and let stand for 30 minutes in the fume hood. If the srong yellow colour of the diazomethane does not persist then repeat the methylation as required.
5. In a fume hood, remove the cap and evaporate the sample to near dryness under nitrogen.
6. Add exactly 2.00 ml hexane and dissolve.
7. For effluent samples, proceed to Step 9. For sediment and biota samples proceed to Step 8.
8. Quantitatively transfer the sample to a Florisil column and clean up using the following procedure:

   Calibrate each batch of Florisil with methylated CP standard to assure complete recovery. This procedure below assumes that all the methylated CP's are eluted with 150 ml of hexane.
   - the column used is 26 cm x 20 mm I.D. with a sintered disk and a teflon stopcock. Note that the packed column should be used as soon as possible.
   - add 70 ml of hexane then 20 g of 2% Florisil pre-slurried with hexane to the column.
   - add 1 cm of heat-treated Na_2SO_4 to the top of the column.
   - pre-rinse the column with 2 x 50 ml portions of hexane.
   - just before exposure of the Na_2SO_4 layer, add the sample and then elute with 2 x 75 ml hexane.
   - collect the extract in 250-ml rotary evaporator flask.
   - add 1 ml of iso-octane as a keeper then evaporate (water temperature less than 35 C) to about 0.5 ml.
   - quantitatively transfer the extract to a 2 ml volumetric with hexane and make up to volume.

contd. ...

PROCEDURE - COMMON TO ALL EXTRACTS (ctd.)

9. Clean Up Procedure Using Activated Copper, conc. Sulfuric Acid and Mercury.
   - add 2 ml of sample and 200 mg activated copper to a 7-ml vial.
   - seal the vial and ultrasonicate the sample for 60 minutes.
   - centrifuge at 2000 rpm for 10 minutes.
   - decant the sample into a clean 7-ml vial.
   - add 1 ml of conc. H_2SO_4, vortex mix for 1 minute then centrifuge.
   - repeat the above treatment until the acid layer is colourless then transfer the sample to a clean vial.
   - add 2 drops of mercury to the extract, seal the vial and ultrasonicate for 30 minutes.
   - centrifuge and decant the sample into a clean 7-ml vial. Repeat the procedure as necessary.
10. The sample is now ready for GC analysis. Use the autosampler to inject 2 ul of standards and samples on Channel A, the "primary" column.
11. Gas Chromatograph Conditions:

Oven Temp:	150 C	Detector Att:	2^(6)
Inlet Temp:	220 C	Carrier gas:	95% argon/5% methane @ 60 ml/min
Detector Temp:	325 C		
Slope Sens:	0.20		
Stop Time:	25 min		

12. Comparison of retention times on Channel B, the "secondary" column, is used to confirm identification, if required.

CALCULATIONS & DATA PROCESSING

The chlorinated phenol concentration is determined by direct comparison of the peak area with a standard, providing the sample and standard are within the linear range of the GC detector. Results are reported to three significant figures.

[CONC] = {[A][C][D][E][F]} / [B] units

A = Sample Peak Area/ul Injected
B = Standard Peak Area/ul Injected
C = Conc. of Standard in ug/ml
D = Volume of hexane extract made up to
E = Dilution Factor (if any)
F = for Effluents = [1000]/[Volume Sample extracted - in ml]
= for Sediments = [1]/[Dry Wt. Sample extracted - in g]
= for Biota = [1]/[Received Wt. Sample extracted - in g]

Reporting Units = for Effluents = ug/l
= for Sediments = ug/g
= for Biota = ug/g [W]

contd. ...

CALCULATIONS & DATA PROCESSING (ctd.)

The concentration of the CP's are entered in the laboratory database by using "RENT" software and entering the following Package (Billing) Code:

CHLOROPHENOLS:	CP	Procedure 800 (Effluents) Procedure 801 (Sediments, Biota)

Result code(s) for the specific chlorophenol isomers will be created automatically as required.

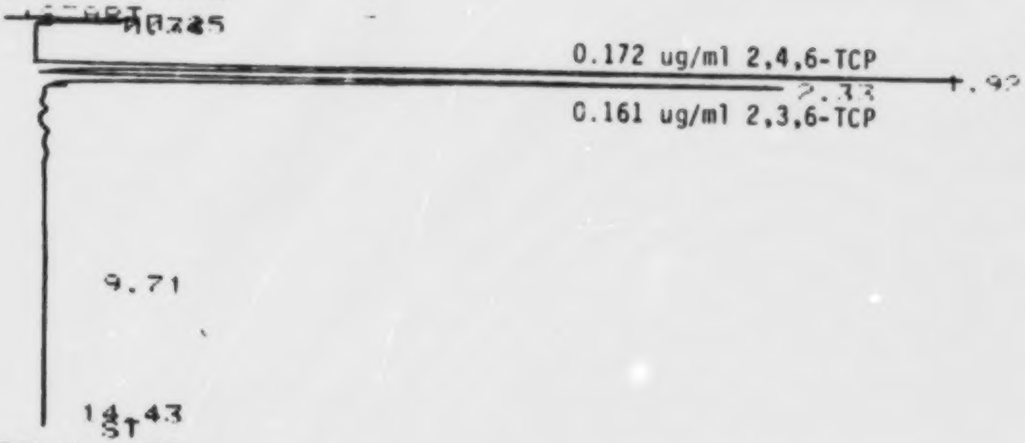
REFERENCES

[1] L. Rudling, Water Research, 4, P533-537, (1970).
[2] G.R. Higginbotham, J. Ress, and A. Rockne, JAOAC, 53, P673-676, (1970).
[3] A.S.Y. Chau and J.A. Coburn, JAOAC, 57, P389-393, (1974).
[4] L.L. Lamparski et al., Gas Liquid Chromatographic Determination of Chlorinated Benzene and Phenols in Selected Biological Matrices, Anal. Chem., Vol. 63, No. 1, (1980).
[5] Tetra Tech, Inc. Recommended Protocols for Measuring Organic Compounds in Puget Sound Sediment and Tissue Samples. Prepared for U.S. E.P.A., Final Report TC-3991-04, December, 1986.

REVISION HISTORY

Version 2.2	FEB/88	Soxhlet extraction procedure deleted.
2.1	MAY/87	Shaker extraction procedure added. Diazomethane now generated from Diazald. Acidification of effluents with 2N H2SO4.
2.0	JULY/86	Parallel procedures for CP's and PCB's. Methylation procedure changes. Confirmation GC column added.
1.1	APR/85	Alkaline pre-extraction of effluents deleted. Solvent and sample preparation changes.
1.0	-	Original Method in 1979 manual.

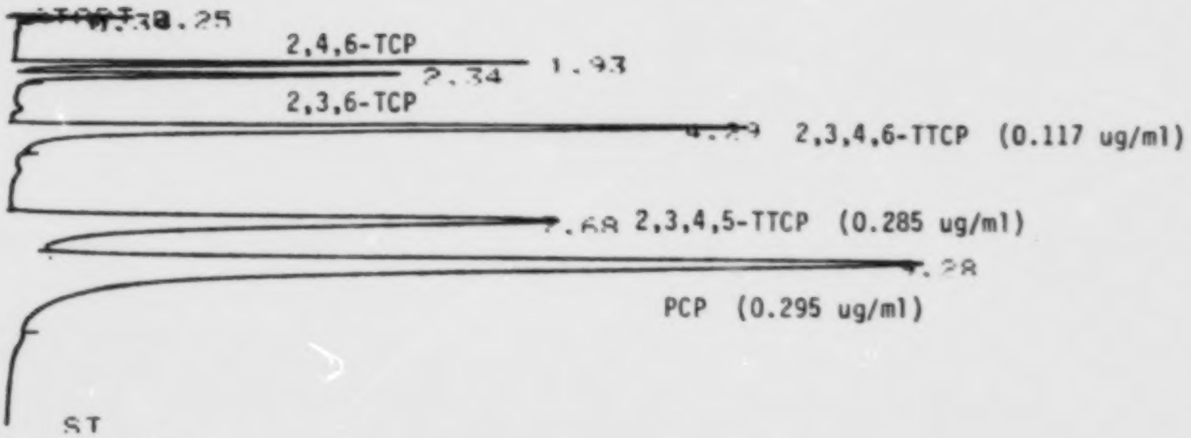
```
TEMP1         150  150
TIME1          99.9
INJ TEMP     220   220
FID TEMP     325   325
AUX TEMP     325   326
OVEN MAX     250

CHT SPD         0.50
ATTN 2↑         7
AUX SGNL      0
SLP SENS        0.00
AREA REJ     100
FLOW  A       62
FLOW  B       61
OPTN          12
15.0  STOP
CHT SPD 5 @
AUX SGNL A  @
AUX SGNL ZERO
ATTN 2↑ 2 2 @
   START CHT
      ATTN 2↑ 7 @
                    STOP


CHT SPD . 5 @
CHT SPD . 4 @
```



```
BOTTLE    32
** 5830A
AREA %

   RT                AREA        AREA %

  0.25               4490         2.266
  0.34               2742         1.384
  1.92              98160        49.533
  2.33              92780        46.818

                   XF:   1.0000 E+ 0
```

Normal Operating Conditions for Running Chlorophenols on a 6%-210, 4%-101 packed column (Channel A) on The HP packed column Gas Chromatograph (Auto-Sampler Mode)

```
TEMP1       150  150
TIME1        99.9
INJ TEMP   220  220
FID TEMP   325  325
AUX TEMP   325  325
OVEN MAX   250

CHT SPD      0.40
ATTN 2↑     10
AUX SGNL     A
SLP SENS     0.00
AREA REJ 1000
FLOW  A     60
FLOW  B     60
OPTN        12
25.0  STOP
```



```
      ST
BOTTLE    34
** 58300
AREA %

   RT            AREA        AREA %

  0.25           5516         0.522
  0.34           1172         0.111
  1.93          54850         5.189
  2.34          47000         4.446
  4.29         179100        16.942
  7.68         243600        23.043
  9.28         525900        49.748

            MF:   1.0000 E+ 0
```

APPENDIX IV

Environment Canada method for TCMTB analysis

Environment Canada
Conservation and Protection

Laboratories
Pacific & Yukon Region

2-(Thiocyanomethylthio)benzothiazole V1.2 March, 1990 SAPLC

High Performance Liquid Chromatography

SCOPE AND APPLICATION

This method is applicable to the determination of 2-(Thiocyanomethylthio)benzothiazole (TCMTB) and the metabolites Benzothiazole (BT), 2-Mercaptobenzothiazole (2MBT) and 2-Methylmercaptobenzothiazole (MMBT) in effluents and sediments. The TCMTB, BT, 2MBT, and MMBT detection limits in effluents are 1, 10, 25, and 5 ug/l, respectively. Sediment detection limits are currently under evaluation.

SAMPLE HANDLING AND PRESERVATION

Note: All glassware, aluminum foil and sampling equipment that comes in contact with the sample must be acetone rinsed and heat-treated at 325 C for a minimum of 12 hr.

Samples should be collected in an all glass system and stored at 4 C or just above the freezing point. If Teflon lined caps are not available for the sampling containers then heat-treated aluminum foil must be used as a liner. At least 1 litre of sample should be collected.

SUMMARY OF METHOD

The sample is first extracted with methylene chloride then the extract is filtered, concentrated and the residue dissolved in acetonitrile. The sample is then analysed for TCMTB and its metabolites MBT, MMBT and BT using High Performance Liquid Chromatography (HPLC).

METHOD PERFORMANCE

1. Five sample replicates yielded a 7.5 Average % Standard Deviation.
2. A DI water solution spiked with TCMTB at the 20 ng/l gave the following average recovery with a Standard Deviation of 7.40 over 5 replicates.

Compound	Actual value (ng)	Found value (ng)	% Recovery
TCMTB	2.8	2.7	95.9%

3. The linear coefficient of correlation for each TCMTB standard was better than 0.996 over a 1 - 43 ng Injection Solution range (5 ul injection).

contd. ...

INTERFERENCES

1. Interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/ or elevated baselines in HPLC chromatograms. All materials must be routinely demonstrated to be free from interferences under the conditions of analysis.
2. Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled.
3. Store standards at 4 C and protect from light. Stock standards should be checked frequently for signs of degradation.
4. Compounds co-eluting with TCMTB and absorbing UV at 280 nm are potential sources of interference.

SAFETY

TCMTB should be treated as a potential health hazard. Exposure should be kept at the lowest level possible. Use well vented fume hoods and wear protective clothes and gloves at all times when using these chemicals.

APPARATUS

All glassware must be washed with heavy-duty soap, rinsed with distilled water, rinsed with acetone and then heat-treated at 325 C for at least 12 hours prior to use. The glassware must be stored in an area protected from chemical contamination.

1. Graduated cylinders, 500-ml.
2. Boiling flasks, 500-ml.
3. Separatory funnels, 1-litre.
4. Vials, 7-ml. with foil cap liners.
5. Volumetric flasks, 2-ml, 10-ml.
6. Rotary evaporator.
7. Centrifuge.
8. Glass columns, 26 cm x 2.0 cm, with sintered glass frits and teflon stopcocks.
9. Visiprep Solid Phase Extraction Vacuum Manifold, Supelco #5-7030.
10. Supelclean Solid Phase Filtration Tubes, Supelco #5-7026.
11. Buchner funnel, 5.0 cm.
12. Vacuum adapter, 24/40.
13. GF/C filter paper, 4.5 cm.
14. Burrel wrist-action shaker.

contd. ...

APPARATUS (ctd.)

15. High performance liquid chromatograph, Hewlett-Packard 1090 equipped with diode-array detector and temperature controlled autosampler. HPLC column, Hewlett Packard, ODS-HYPERSIL(C18), 5um, 200mm x 2.1 mm i.d.(microbore).
16. Autosampler vials and caps, 2-ml.

REAGENTS

1. Methanol, Burdick & Jackson HPLC grade, filtered through Nylon 66 membrane filter (0.2 u x 47 mm diameter).
2. Methylene chloride, Burdick & Jackson HPLC grade.
3. Acetonitrile, Burdick & Jackson HPLC grade, filtered through Nylon 66 membrane filter (0.2 u x 47 mm diameter).
4. Deionized water, filtered through Nylon 66 membrane filter (0.2 u x 47 mm diameter).
5. TCMTB (99.3%), Buckman Laboratories, P.O. Box 8305, Memphis, Tennessee, 38108.

PROCEDURE - GENERAL

1. Blanks are required for each new batch of reagents.
2. Duplicates and spiked samples must be run on a routine basis.
3. Standards should be injected frequently as a check on the stability of operating conditions.

PROCEDURE - EXTRACTION OF EFFLUENTS

1. Add 500 ml of the shaken sample to a 1-litre separatory funnel.
2. Extract TCMTB and metabolites by adding 75 ml methylene chloride and vigorously shaking the separatory funnel for 2 minutes.
3. Extract twice more with 75 ml of methylene chloride, draining the solvent fraction through a funnel plugged with glass wool and 5 g Na2SO4 into a 500-ml round bottom flask after each extraction.
4. Rotary evaporate the solvent to dryness then quantitatively transfer the residue to a 2-ml volumetric flask with acetonitrile by filtering through a Supelco filter tube and filtration apparatus.
5. The sample is now ready for analysis.

PROCEDURE - EXTRACTION OF SEDIMENTS

1. Air dry approximately 50 g of the sample overnight. Weigh 25 g of the air-dried sample into a 100-ml heavy-walled shaker tube, add 50 ml of methylene chloride and shake the sample on a wrist-action shaker for 2 hours.

contd. ...

PROCEDURE - EXTRACTION OF SEDIMENTS (ctd.)

2. Vacuum filter the methylene chloride extract through a Buchner funnel with a vacuum adapter using Whatman #1 filter paper and collecting in a 500-ml round bottom flask. Rinse the shaker tube twice, each with 25 ml of methylene chloride and filter as above.
3. Add approximately 5 g of heat treated sodium sulfate to the flask, then cover and let the extract stand for 30 min.
4. Transfer the extract to a clean 500-ml round-bottom flask and rotary evaporate to dryness.
5. Quantitatively transfer the residue to a 2-ml volumetric flask with acetonitrile by filtering through a Supelco filter tube and filtration apparatus.
6. The sample is now ready for analysis.

PROCEDURE - HPLC START-UP

NOTE: All control of the HPLC should be from the HP 300 computer.

1. Ensure the HP 1090 mainframe is turned on then turn on the computer.
2. Check the solvent and distilled water reservoirs. Use freshly deionized water daily. Turn on Helium for solvent degassing.
3. Turn on the circulating bath for cooling of the autosampler.
4. Empty the waste solvent reservoir daily.
5. Check for sufficient printer paper.
6. Once computer has been booted it will show that you are at TOP LEVEL. Press the (F2) soft key, <DATA ACQUIS>. To move to different areas on the screen use the <TAB> key.

PROCEDURE - SET UP OF HPLC SEPARATION PARAMETERS

1. Once at <DATA ACQUIS> level press (F6) soft key, <EDIT PARAMS>. To get the TCMTB conditions press (F1), <LOAD PARAMS>, press <?><RETURN>, and (F1), <SELECT ITEM> TCMTB.A. This loads all the liquid chromatograph and diode array detector conditions required to separate TCMTB and its metabolites BT, 2MBT, and MMBT. Press (F8), <EXIT>.
2. Press (F7), <MORE KEYS>, press (F3), <PUMP ON>, press (F7), <MORE KEYS>, Press (F3), <MONITOR>. The wavelength screen will have spurious peaks and must be balanced by pressing (F6), <BALANCE> key. A flat baseline should then be obtained. DO NOT PROCEED UNTIL THIS IS CORRECT. Then press (F8), <EXIT> and then (F8), <QUIT>.
3. Once at TOP LEVEL again, press the (F5), <SEQUENCE> soft key. The cursor key will be at the sequence table line, press <?><RETURN> and (F1), <SELECT ITEM> T1.R. Tab down to Sample Table file name, press <?><RETURN> and (F1), <SELECT ITEM> T1.S. Tab down to Data file name and set your

contd. ...

3.(ctd.)
Data file as follows: If running multiple samples and injections the file name is replaced with _____00A. The first 5 characters you can select as the descriptor. The last 3 characters (00A) are used by the Chem Station as a counter for the vial and injection numbers.

ex. _____00A.D - Vial #0 (00) injection number 1(A).
_____02A.D _ Vial #2 (02) injection number 1(A).

Use an "A" for the fifth character of the Sequence run for automated runs. For routine samples the last four characters of the Laboratory Sample Number should be used (ex. 0833AxxA.D). The counter sequence will identify the sub-sample. When running an unattended analysis on the HPLC start at Sequence Line A then tab down to operator name and fill in as appropriate.

4. Press (F1) soft key, <EDIT SEQ FILE>, then <?><RETURN> and (F1), <SELECT ITEM> TCMTB.M. Set the Range with how many samples are to be run and where they are situated in the auto-sampler (i.e. Range 00:30 Rep 1 represents sample vial 1 to sample vial 30 with one injection). If any existing sequence files are present they can deleted by using the space bar and erasing the existing files. Press (F8), <EXIT> from Seq file.
5. Press (F2) soft key, <EDIT SAMPL TBL>. This table identifies every sample and how it is going to be analyzed (i.e. either a sample or calibration standard). Start by indexing the sample number, sample identification and then pressing (F5), <MORE INFO> to give the sample more identification. In this section you put in any dilution factor incorporated in the sample, is it a calibration sample or not. If it is a calibration standard, at what level is it at with your existing calibration curve. If it is a recalibration standard you can either replace the level or average the level. Do this for every sample wanted to be analyzed. As in the Sequence file any existing samples in the table can be deleted by "Space Barring" through the Sample table and erasing existing samples.
6. Press (F8), <EXIT>, then press (F6), <CHECK METHODS>. If all is correctly set up you can press (F4), <START SEQUENCE> and unattended run can proceed.
7. Once START SEQUENCE has commenced press (F7), <HOLD METHOD> and check that the DATA ACQUIS FILE and all the conditions are correct. Check that the right vial is being injected. If condition are not met press (F8), <STOP METHOD>, wait for the first run to finish and recheck all parameters.
8. Note that you can check the integrity of your standard curve by re-running standards and "addressing" them in the sample table (see 5.).
9. Press (F8), <RESUME> once everything is correct and run the samples.
10. The REPORT GENERATOR will provide all the quantitation results and report if the sample was a recalibration standard.

contd. ...

PROCEDURE - HPLC ANALYSIS

1. Liquid Chromatograph Conditions:

FLOW: 0.50 ml/min
SOLVENT A: 50% H20
SOLVENT C1: 50% ACETONITRILE
OVEN TEMP: 40.0 C
STOP TIME: 10.0 min
POST TIME: 3.00 min
INJ. VOL.: 10.0 ul

GRADIENT CHANGE: TIME 0.0 min = 50% Acetonitrile.
TIME 10.0 min = 100% Acetonitrile.

DIODE ARRAY DETECTOR CONDITIONS:

SIGNALS: A
SAMPLE: Wavelength = 281nm
Bandwidth = 4nm
REFERENCE: Wavelength = 550nm
Bandwidth = 100nm

Store the spectrum under peak controlled conditions with a threshold of 0.4 mAU, and a peakwidth of 0.100 min.

2. Inject exactly 10.0 ul of the TCMTB Standard Mixes T1 - T4 in the appropriate concentrations and injected on the HPLC for confirmation of individual peak identification by retention time and full diode array library match.

TCMTB and METABOLITES	CONCENTRATION (ng/ul)			
	T1	T2	T3	T4
TCMTB	132.0	66.0	33.0	6.6
2-Mercaptobenzothiazole	118.0	59.0	29.5	5.9
Benzothiazole	115.0	57.5	28.8	5.8
MMBT	154.0	77.0	38.5	7.7

3. Inject exactly 10.0 ul of each sample and collect data.

contd. ...

CALCULATIONS AND DATA PROCESSING

[SAMPLE CONC.] = {[INJECTION SOLUTION CONC.] X [A] X [B] X [C]}

INJECTION SOLUTION CONC. = from Quantitation Report, in ng/ul
A = Final Volume of Injection Solution, in ml.
B = Dilution Factors (if any).
C = for Effluent Samples = [1000]/[Volume Sample extracted - in ml]
= for Sediment Samples = [1]/[Dry Wt. Sample extracted - in g]

Reporting Units: for Effluent Samples = ug/l
: for Sediment Samples = ug/g

The concentrations of TCMTB, BT, 2MBT, and MMBT are entered in the laboratory database using "RENT" software and entering "SAPLC" for the Package (Billing) Code.

REFERENCES

[1] Hewlett Packard HP1090 Liquid Chromatograph Fact File Handbook, First Edition, November, 1987.
[2] Stability of TCMTB Anti-Sapstain Solutions, Prepared by B.C. Research, April, 1985.
[3] 2-(Thiocyanomethylthio)benzothiazole in Air, Worker's Compensation Board of British Columbia, 20 Feb., 1985.
[4] Analytical Method of TCMTB (communication), Buckman Laboratories International, Inc., July 6, 1988.
[5] Environmental Dynamics of Benzothiazole-Based Pesticides, B.G. Brownlee, Rivers Research Branch, National Water Research Institute, April, 1989.
[6] Benzthiazoles in estuarine sediments as indicators of street runoff, Robert B. Spies, Brian D. Andresen & David W. Rice Jr., Nature, June 25/87.

REVISION HISTORY

Version 1.2	MAR/90	Change to microbore HPLC column resolves all metabolites analyzed for. ECD-GC and GC/MS procedures no longer required.
1.1	DEC/89	Documentation upgrade, addition of MMBT by ECD-GC & GC/MS.
1.0	NOV/88	New method.

contd. ...

PROGRAM USED BY HPLC COMPUTER SYSTEM (H/P 300 CHEMSTATION) TO IDENTIFY PEAKS FOR PURITY AND LIBRARY MATCH BASED ON SPECTRAL LIBRARY FIT AND RETENTION TIME

```
!------------------------------------------------------------------!
!   Macro for automated spectral library search                    !
!                                                                  !
!     Signal with peaks in z-register.                             !
!                                                                  !
!   AUTOLIB.G Version 4.01                                         !
!      First release.                                              !
!   AUTOLIB.G Version 4.06                                         !
!     If sequence running then no query for input and default      !
!       settings (library is taken from method data file param)    !
!------------------------------------------------------------------!

NAME AUTOLIB
  seqinf              ! sequence info
  CHR ,81
  EX X,Z
  gets header,z
  If Nobjects=0 Then
    Convert A$,"No signals in Z-register, please select first"
    lib_ABORT              ! return to data editor macros
    RETURN
  EndIf
  Strpos "AUTOLIB 4.06 Initialized",AutolibIn$
  if len1=len2 then
    if value=1 then
    else
       INITAULIB     ! first time so initialize variables
  endif
else
       INITAULIB     ! first time so initialize variables
  format merged
  clear
  cl x
  cl y
  cl t

  if seq_run=0 then   ! no method or sequence so ask
    Convert A$,"N"
    Getvar A$,"Change settings (No)"
    strpos "Y",A$
    if value>0 then
```

contd. ...

```
      SETVAR          ! change settings
    endif
    DADlib FILE
  else                ! sequence/method running
    DADlib FILE,,NOQ  ! open default library
  endif
  ex x,z
  ent
  mul 1
  DRAW sigtowin,y
  filestart=xlow
  fileend=xhigh
  ref_time=filestart
  spec filend
  lastspec=scan_num   ! get last spectrum available
  gets header,z
! dychar=charsize/10*(yhigh-ylow)/40
  dychar=charsize/10*(yhigh-ylow)/(40-fieldsize-7)  !???????????
  ysc=yhigh+5*dychar
  if numpeaks=0 then
    INTEGRATE Z
    numpeaks=npeaks
  endif
  PeaksFound=NumPeaks
  If PeaksFound=0 Then
  Convert A$,"No peaks found; please change int params first"
    lib_ABORT
    RETURN
  EndIf

  if 1=2 then
    size=0
    x1=0.5
    while size<20
      annot sigtowin,0,size,0,x1,0.3,"C&P Library search"
      size=size+1
      x1=x1-0.01125
      annot sigtowin,0,size,3,x1,0.3,"C&P Library search"
    endwhile
  endif

  if doprint=1 then
    PRHEAD
  endif
```

contd. ...

```
CurPeak=0
dr sigtowin,y
format merged
while CurPeak<Peaksfound
  CurPeak=CurPeak+1
  MESS "Peak spectra search....                       ",,0,-5
  PEAKS CurPeak,,none,Z,1,st_wave:en_wave
  Crest=Ret_Time
  rol -1
  spec ret_time,st_wave:en_wave
  top_time=ret_time
  if (ret_time-start_time)*(end_time-ret_time)>=0 then

    Convert A$,"Peak#",CurPeak:4:0,"  time:",RET_TIME:7:3
    mes A$,,0,-6

   !--  get the spectr/a/um  --!
    if dopeakpur=1 then
      PEAK_SPEC
    else
      if dosubref=1 then
        if ref_spec<0 then
           PEAK_SPEC
        endif
      endif
    endif

    !-- subtract reference spectrum  --!
    if dosubref>0 then
       SUBREF
    endif

    !-- remove empty objects  --!
    gets header,x
    objs=nobjects
    i=1
    while i<=objs
      gets header,x,i
      if ylow=0 then
        if yhigh=0 then
          extr i
          rol -1
          i=i-1
```

contd. ...

```
     objs=objs-1
    endif
  endif
  i=i+1
endwhile

gets header,x
if nobjects>0 then

  if nobjects=2 then
    if dopeakpur=0 then
      extr
      add
      mul 0.5
   endif
  endif
   SMDERNORM
  gets header,x
  if nobjects>1 then
    dr purtowin,x
    compare ab_thres,0,x,1,x,nobjects
    Convert A$,"Purity:",match:6:1
    ann purtowin,,,,,,A$,xaxis
    dr purtowin,x
    peak_pur=match
    if match>purthres then
      if dosubref>0 then
        if ref_spec<0 then
          extr
          add
          mul 0.5
        else
          ro -1
          spec top_time,st_wave:en_wave
       SUBREF
        endif
      else
        ro -1
        spec top_time,st_wave:en_wave
      endif
       SMDERNORM
    else
```

contd. ...

```
    DADLib SEARCH,SPECTRUM,ab_thres,cor_sh,leftwin:rightwin,x,nobjects
  endif
else
  peak_pur=-1000
endif
DADLib SEARCH,SPECTRUM,ab_thres,cor_sh,leftwin:rightwin,x,1
if doprint=1 then
  write 701,CurPeak:2:0,crest:9:2
  if dopeakpur=1 then
     if peak_pur>-0.1 then
       writereal 701,peak_pur:6:0
    else
      write 701,"   ???"
    endif
 else
    write 701,"   ---"
  endif

  upslope=0
  downslope=0
  DADlib RES,1,x,1
  if entry>0 then
    n_object=1
     gets header,x
    if nobjects>1 then
      upslope=1
    endif
     PRSEAR
    upslope=0
  else
    writeln 701,match:12:0
    endif
    gets header,x
    if nobjects>1 then
      n_object=nobjects
    downslope=1
     PRSEAR
    downslope=0
  endif

  mathfunc trunc,CurPeak/5,x
  if CurPeak-(x*5)=0 then
    writeln 701,""
  endif
endif
```

contd. ...

```
gets header
if nobjects=1 then
  convert A$,""
  DADlib RES,1,x,1
  high_match=match
else
  if peak_pur>purthres then
    convert A$,""
  else
    convert A$,"*"
  endif
  high_match=0
  DADlib RES,1,x,1
  If entry>0 then
    high_match=match
  endif
  DADlib RES,1,x,nobjects
  If entry>0 then
    if Match>high_match then
            high_match=match
  else
  DADlib RES,1,x,1
  high_match=match
    endif
  endif
endif
If entry>0 then
  if high_Match>=searthres then
    DADlib READ,entry,st_wave:en_wave
    convert A$,A$,Comp_name$
    norm 100
    if cor_sh=0 then
    else
      gets header
      trans xlow:xhigh,(xlow+cor_sh):(xhigh+cor_sh)
    endif
    merge

    dr spetowin
    SCREENPRINT 640,500
    mess "Entry#:",entry,0,-5
    mess "high_Match:",Match,20,-5
  else
```

contd. ...

```
          convert A$,A$,"?"
        endif
      else
        convert A$,A$,"?"
      endif
      if adjanot=1 then
        gets header,y
        gets point,start_time:end_time,z
        ymax=y+0.5*dychar
      endif
      if ymax+((fieldsize+5)*dychar)>ysc then
        ysc = ymax+((fieldsize+5)*dychar)
      endif
      AN sigtowin,anotrot,charsize,3,crest,ymax,A$[1:fieldsize],DATA,1
    endif
  endif
endwhile
Convert A$,"Library : ",lib_name$
AN sigtowin,,,6,0.15,0.90,A$
AN sigtowin,,,6,0.15,0.94,"Results of spectral library search"
Convert A$,"Match > ",searthres:3:0
an sigtowin,0,10,3,0.02,0.01,A$
clear
gets header,y
dr 3,y,,ylow:ysc*0.8
if doprint=1 then
  write   701,"-------------------------------------"
  writeln 701,"-------------------------------------"
  writeln 701,""
  makeprint
endif
CL
CHR ,81
EX X,Z
EV TCMTB.E
START SET
INT Z
DRAW 3,Z
GETS Z
DRAW 3,Z,,0:YHIGH*1.2
PAGES 1
DRAW PRINTER:,Z,,0:YHIGH*1.2
QUIT
RETURN
```

contd. ...

```
NAME MAKEPRINT
  win 4,0:1,0:1
  clear
  gets header,y
  dr 4,y,,ylow:ysc
  screenprint 640,500

  Clear
  dr 3,y,,ylow:ysc
  return
NAME MAKEPLOT
  win 4,0:1,0:1
  clear
  gets header,y
  dr 4,y,,ylow:ysc
  redraw 705

  Clear
  dr 3,y,,ylow:ysc
  return
!--------------------------------------!
!                                      !
!--------------------------------------!
NAME CHECKWAVE
  scan 1
  rol -1
  gets header,t,1
  if en_wave<=st_wave then
    st_wave=xlow
    en_wave=xhigh
  else
    if st_wave<xlow then
      st_wave=xlow
    endif
    if en_wave> >xhigh then
      en_wave=xhigh
    endif
    if en_wave<=st_wave then
      st_wave=xlow
      en_wave=xhigh
    endif
  endif
  RETURN
```

contd. ...

```
!------------------------------------!
! change settings                    !
!------------------------------------!

NAME SETVAR
  if leftwin>-0.01 then
    Convert A$,"Yes"
  else
    Convert A$,"No"
  endif
  Convert B$,"Library time search window (",A$,") >"
  getvar A$,B$
  strpos "Y",A$
  if value>0 then
    if leftwin<0 then
      leftwin=5
    endif
    Convert A$,"Search window (% of ret. time) (",LeftWin:0:1,") >"
    Getvar LeftWin,A$
    rightwin=leftwin
  else
    leftwin=-1
    rightwin=-1
  endif
  if dosubref=1 then
    Convert A$,"Yes"
  else
    Convert A$,"No"
  endif
  Convert B$,"Subtract background (",A$,") >"
  getvar A$,B$
  strpos "Y",A$
  if  value>0 then
    dosubref=1
    value=-1
    while value<0
      if ref_spec=0 then
        Convert A$,"Base"
      else
        if ref_spec<0 then
          Convert A$,"Apex"
        else
          Convert A$,"Ref"
```

contd. ...

```
      endif
    endif
    Convert B$,"Type of background, Ref/Base/Apex (",A$,") >"
    getvar A$,B$
    strpos "BA",A$
    if value>0 then
      ref_spec=0
    else
      strpos "AP",A$
      if value>0 then
        ref_spec=-1
      else
        strpos "RE",A$
        if value>0 then
          if ref_spec<0 then
            ref_spec=0.01
          endif
          convert A$,"Give time of reference spectrum (",ref_spec:7:3,") >"
          Getvar ref_spec,A$
        else
          mess "unknown type"
        endif
      endif
    endif
  endwhile
else
  dosubref=-1
endif
 CHECKWAVE
Convert A$,"Wavelength range from (",st_wave:0:1,") >"
Getvar st_wave,A$
Convert A$,"Wavelength range to (",en_wave:0:1,") >"
Getvar en_wave,A$
if do smooth=1 then
  Convert A$,"Yes"
else
  Convert A$,"No"
endif
Convert B$,"Smooth before search (",A$,") >"
getvar A$,B$
strpos "Y",A$
if  value>0 then
```

contd. ...

```
  dosmooth=1
  Convert A$,"Number of smooth points, >5 (",smoothfac:0:0,") >"
  Getvar smoothfac,A$
else
  do smooth=0
endif
Convert A$,"Match threshold for library (",SearThres:0:0,") >"
Getvar SearThres,A$
if dopeakpur=1 then
  Convert A$,"Yes"
else
  Convert A$,"No"
endif
Convert B$,"Peak purity check (",A$,") >"
getvar A$,B$
strpos "Y",A$
if  value>0 then
  dopeakpur=1
  Convert A$,"Match threshold for purity (",purThres:0:0,") >"
  getvar purthres,A$
else
  dopeakpur=0
endif
if doprint=1 then
  Convert A$,"Yes"
else
  Convert A$,"No"
endif
Convert B$,"Printout of results (",A$,") >"
getvar A$,B$
strpos "Y",A$
if value>0 then
  doprint=1
else
  doprint=0
endif
RETURN
```

contd. ...

```
!--------------------------------------!
! print heading                        !
!--------------------------------------!
NAME PRHEAD
    tof
    writeln 701,""
    write   701,"
    writeln 701,"C&P automated Library search macro (rev 4.06)#27&d@#15  ------"
!   writeln 701,"#10"
!   screenprint 640,500,printer:
    writeln 701,"#10"
    writeln 701,"Library file                   : ",FILE_L$
    writeln 701,"           name                : ",lib_name$,#10
    writeln 701,"Match threshold                : ",Searthres:0:0
    if cor_sh>0 then
      writeln 701,"WL calibration cor.    : ",cor_sh:0:1," nm"
    endif
    write 701,"reference spectrum    : "
    if dosubref=1 then
      if ref_spec= then
        writeln 701, "Baseline"
      else
        if ref_spec<0 then
          writeln 701,"Apex"
        else
          writeln 701,ref_spec:0:2," min"
        endif
      endif
    else
      writeln 701,"None"
    endif
    if der_order>0 then
      writeln 701,"Order of derivative    : ",der_order:0:0
    endif
    write 701,"Smooth                : "
    if dosmooth=1 then
      writerealn 701,smoothfac:0:0
    else
      writeln 701,"None"
    endif
     CHECKWAVE
    writeln 701,"Wave range                 : ",st_wave:0:1," to ",en_wave:0:1," nm"
    write 701,"Time window           : "
    if leftwin<0 then
```

contd. ...

```
      write 701,"None"
    else
      writeReal 701,leftwin:0:1
    endif
    if leftwin=rightwin then
    else
      write 701," to "
      if rightwin<0 then
        write 701,"None"
      else
        writeReal 701,rightwin:0:1
      endif
     endif
     writeln 701," %"
!   writeln 701,"Absorbance threshold  : ",ab_thres:0:1," %"
    write 701,"Peak purity threshold : "
    if dopeakpur=1 then
      writeRealn 701,purthres:0:0
    else
      writeln 701,"none"
    endif

  writeln 701,"#10#10File     : ",file_d$
  writeln 701,"Date     : ",inj_date$
  writeln 701,"Name     : ",data_name$
  writeln 701,"Info     : ",misc_info$
  writeln 701,"Operator: ",operator$
  if inj_num>0 then
    writeln 701,""
    write   701,"Sequence index : "seq_index:0:0
    writeln 701,"  vial : ",bottle:0:0," replicate : ",inj_num:0:0
  endif

  write   701,"#10#10----- Sample ------     #27&dD#14Match#27&d@#15"
  write   701,"     ----------------"
  writeln 701," Library --------------------"
  writeln 701,"Peak#---------------------------------------------"
  writeln 701,"--------------------------"
  RETURN !PRHEAD!
```

contd. ...

```
!-----------------------------------!
! ads a spectrum to x register      !
!-----------------------------------!
NAME AD_SPEC
  if ret_time<start_time then
    clear x
  else
    if ret_time>end_time then
      clear x
    endif
  endif
  gets header
  if nobjects=0 then
    ro -1
  else
    Convert Z$,header$
    gets header,y
    if nobjects=0 then
      ro -1
       ex x,t
    else
      i=1
      while i<=nobjects
        gets header,y,i
        strpos header$,Z$
        if value=1 then
          if len1=len2 then
            value=0
            i=nobjects
          else
            value=1
          endif
        else
          value=1
        endif
        i=i+1
      endwhile
      if value=0 then
        ro -1
      else
        merge
      endif
    endif
 endif
 RETURN
```

contd. ...

```
!-----------------------------------!
! get up/top/down spectra           !
!-----------------------------------!
NAME PEAK_SPEC
  cl x
  Spec Crest-(Peak_Width)/2,st_wave:en_wave
   AD_SPEC
  Spec Crest,st_wave:en_wave
   AD_SPEC
  Spec Crest+(Peak_Width)/2,st_wave:en_wave
   AD_SPEC
  RETURN !PEAK_SPEC!

!-----------------------------------!
! find baseline spectrum.           !
!-----------------------------------!
NAME GETBSPEC
  spec crest,st_wave:en_wave
  found=0
  while found=0
    if value=69 then
      ref_time=ret_time
      found=1
    else
      rol -1
      if scan_num>=lastspec then
        mes "No base spectrum found, set to:",ref_time,0,-5
        spec ref_time,st_wave:en_wave
        found=1
      else
        scan scan_num+1,st_wave:en_wave
      endif
    endif
  endwhile
  RETURN !GETBSPEC!
```

contd. ...

```
!------------------------------------------!
! subtract ref spec                        !
!------------------------------------------!
NAME SUBREF
  if ref_spec=0 then
    GETBSPEC
  else
    if ref_spec<0 then
      mul -1
      Spec Crest,st_wave:en_wave
      mul -1
    else
      Spec ref_spec,st_wave:en_wave
      ref_time=ret_time
    endif
  endif
  subtr
  SCREENPRINT 640,500

  RETURN !SUBREF!

!------------------------------------------!
! subtract ref spec                        !
!------------------------------------------!
NAME PRSEAR
  DADlib RES,1,x,n_object
  if entry>0 then
    il=1
    doit=1
    while doit>0
      DADlib RES,il,x,n_object
      doit=0
      if entry>0 then
        DAdlib Read,entry
        rol -1
        if il=1 then
          doit=1
        endif
        if match>searthres then
          doit=1
        endif
      endif
      if doit>0 then
        if il=1
```

contd. ...

```
        gets header,x,n_object
        strpos "(",header$
        Convert time,header$[3:(value-1)]
        if upslope=1 then
          writeln 701,""
          write 701,time:11:2,"          up"
        else
          if downslope=1 then
            write 701,time:11:2,"        down"
          else
            write 701,"     "
          endif
        endif
      else
        write 701,"                      "
      endif
      write 701,match:07:0,Ret_time:10:2,entry:5:0

      convert A$,"    "
      if match>searthres then
        if il=1 then
          Convert comp_name$,"#27&dD#14",comp_name$,"#27&d@#15"
          if upslope=1 then
            convert A$,"  * "
          endif
          if downslope=1 then
            convert A$,"  * "
        endif
      endif
    endif
      writeln 701,A$,comp_name$
    endif
    il=il+1
  endwhile
endif
RETURN
```

contd. ...

```
!-----------------------------------!
!  get derivative/smooth/norm spectra  !
!-----------------------------------!
NAME SMDERNORM
  if der_order>0 then
    i1=1
    while i1<=der_order
      derivative
      i1=i1+1
    endwhile
  else
    if dosmooth=1 then
      smooth smoothfac
    endif
  endif
  norm 100
  RETURN !SMDERNORM!
!-----------------------------------!
! ABORT                             !
!-----------------------------------!
Name lib_ABORT
  mes A$,,0,-5
  if seq_run=yes then
    RETURN                  ! return to caller
  endif
                            ! stop

!-----------------------------------!
! initializes the macro             !
!-----------------------------------!
NAME INITAULIB
  dosubref=-1
  ref_spec=-1           ! no reference spectrum
  der_order=0           ! order of derivative
  dopeakpur=1           ! peak purity check (1=yes 0=no)
  adjanot=1             ! adjust compound nam
  smoothfac=11          ! smooth factor
  doprint=1             ! printout of results (1=yes, 0=no)
  fieldsize=15          ! annotation field size
  charsize=13           ! annotation character size
  anotrot=90            ! annotation string rotation
  sigtowin=1
  spetowin=2
  purtowin=4
```

contd. ...

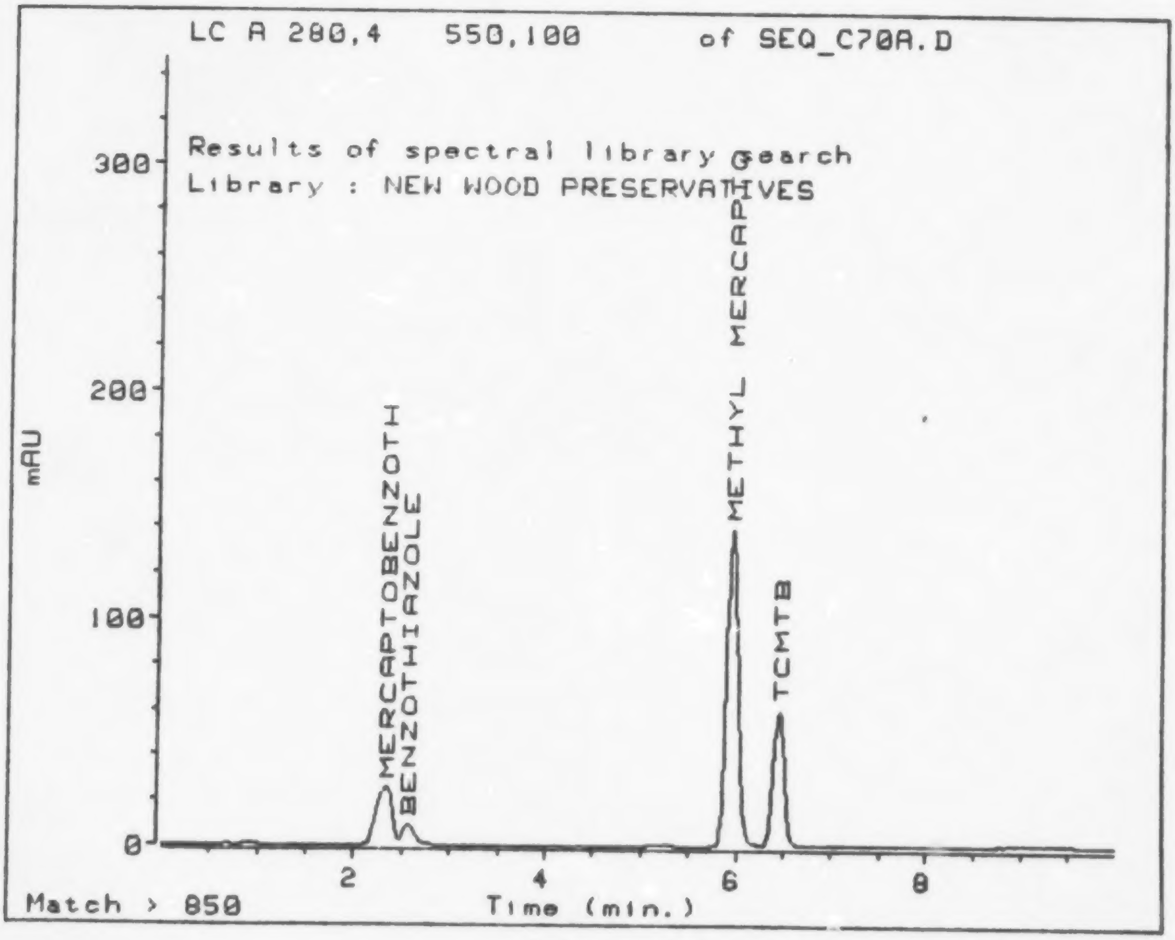
```
  seqinf
  if seq_run=0 then    ! Take the values from DATA EDITOR
    purthres=matchthres! purity threshold (above this threshold peak is pure)
    leftwin=leftwin    ! search window size 5 % of rete
    st_wave=startwave  ! start wavelength range
    en_wave=endwave    ! end wavelength range
    cor_sh=waveshift   ! wavelength calibration shift for spectral library
    ab_thres=absthres  ! mAU threshold for spectral library
    searthres=matchthres ! Match factor above which peak is identified.
  else                 ! set values
    purthres=500       ! purity threshold (above this threshold peak is pure)
    leftwin=20         ! search window size 5 % of retention time
    rightwin=15        ! when <0 then no search window
    st_wave=0          ! start wavelength range
    en_wave=0          ! end wavelength range
    cor_sh=0           ! wavelength calibration shift for spectral library
    ab_thres=2         ! mAU threshold  peak is identified.
    searthres=500      ! Match factor above which peak is identified.
  endif

  Convert AutolibIn$,"AUTOLIB 4.06 Initialized"  ! set autolib is initialized.

  RETURN

!--------------------------------------!
! removes the macro                    !
!--------------------------------------!
NAME REMUSERMAC
  RemAUTOLIB,MakePrint,MakePlot,InitAuLib,CheckWave,SetVar,PrHead,Ad_Spec
  Rem Peak_SPec,GetBspec,SubRef,PrSear,SmDerNorm,Lib_Abort
  RETURN

!--------------------------------------!
!                                      !
!--------------------------------------!
NAME DOUSERMAC
   VM
  OLD_VM=VM_MEM
  VM ,60000
   AUTOLIB
  VM ,OLD_VM
   DISPSTATUS=1
   REMUSERMAC
  RETURN
```

contd. ...

HPLC CHROMATOGRAM AND LIBRARY SEARCH RESULTS FROM A STANDARD MIX OF TCMTB AND METABOLITES:

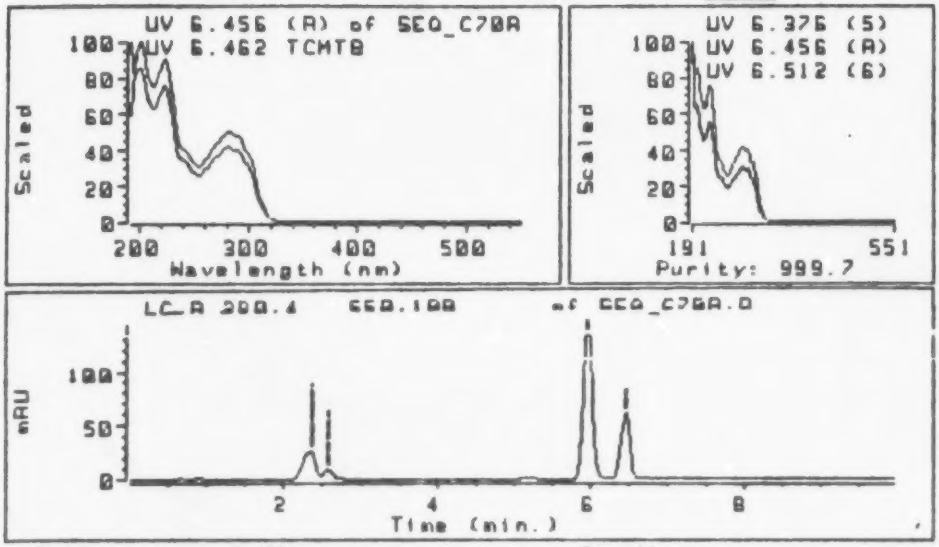
AUTOMATED LIBRARY FINAL OUTPUT

```
----- Sample ------      Match      ----------------- Library --------------------
Peak#  Time  Purity                 Time  Entry#      Name
----------------------------------------------------------------------------------
  1    2.34   871          999      2.24    3     MERCAPTOBENZOTHIAZOLE
  2    2.57  1000          999      2.53    4     BENZOTHIAZOLE
  3    5.96  1000          901      5.98    1     METHYL MERCAPTOBENZOTHIAZOLE
  4    6.46  1000          922      6.46    2     TCMTB
----------------------------------------------------------------------------------
```



AUTOMATED LIBRARY SEARCH MACRO

```
4     6.46  1000          922       6.46     2     TCMTB
```



```
Peak#  4  time:  6.456                          T: null.
Entry#: 2.0000earch.high_Match: 922.48          Z: LC A 280,4   550,100
                                                Y: LC A 280,4   550,100
                                                X: Set of  2 UV
```

TCMTB

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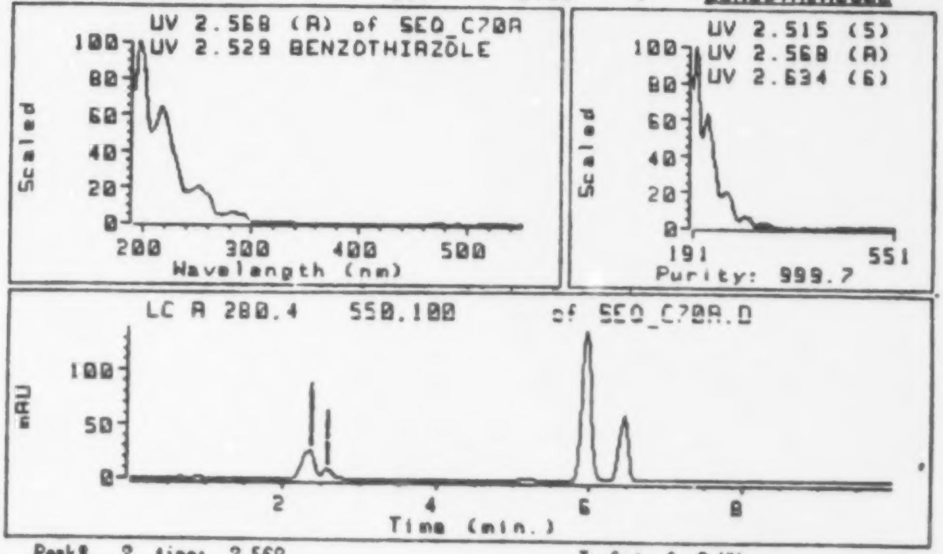
AUTOMATED LIBRARY SEARCH MACRO

```
2       2.57  1000            999        2.53     4     BENZOTHIAZOLE
```



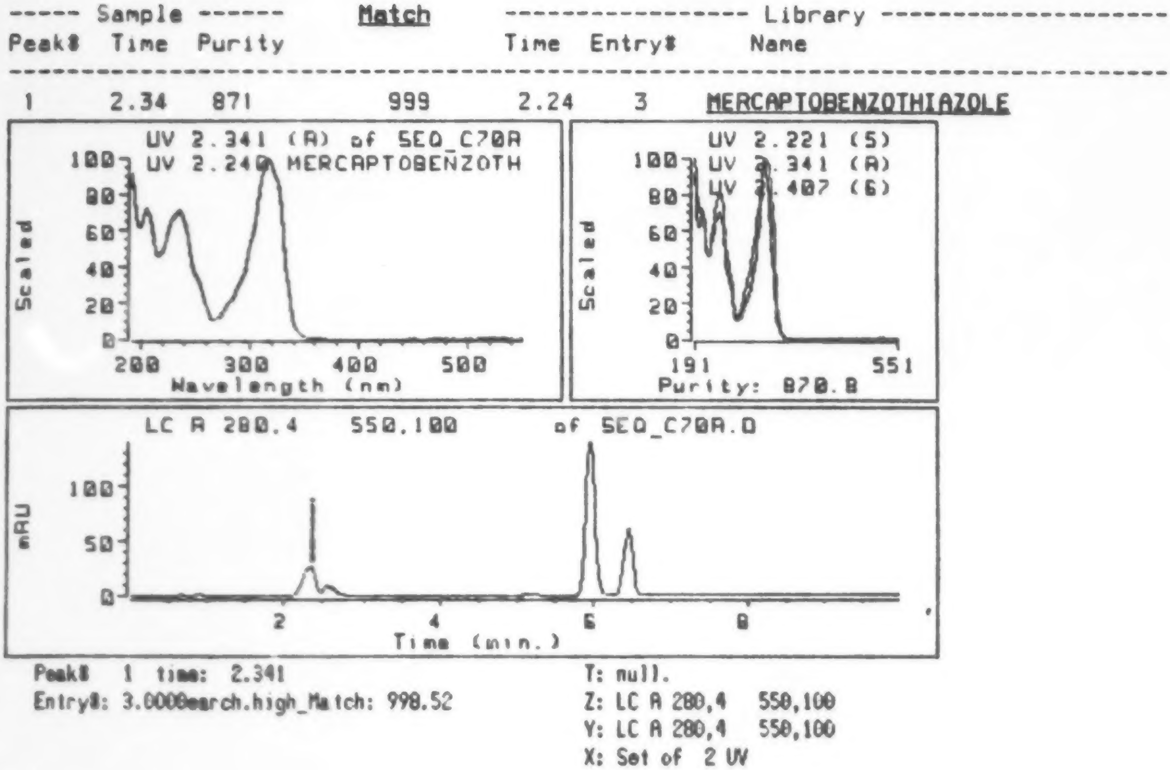
```
Peak#  2  time:  2.568
Peak spectra search.....
                                        T: Set of  2 UV
                                        Z: null.
                                        Y: LC A 280,4   550,100
                                        X: LC A 280,4   550,100
```

Benzothiazole

Environment Canada   Environnement Canada

Canada

AUTOMATED LIBRARY SEARCH MACRO



```
----- Sample ------      Match      ----------------- Library --------------------
Peak#  Time  Purity                 Time  Entry#      Name
-----------------------------------------------------------------------------------
 1      2.34   871          999      2.24    3      MERCAPTOBENZOTHIAZOLE
```

```
Peak#   1  time:  2.341                            T: null.
Entry#: 3.0000earch.high_Match: 998.52             Z: LC A 280,4   550,100
                                                   Y: LC A 280,4   550,100
                                                   X: Set of  2 UV
```

2-Mercaptobenzothiazole

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tcmtb-v1.2

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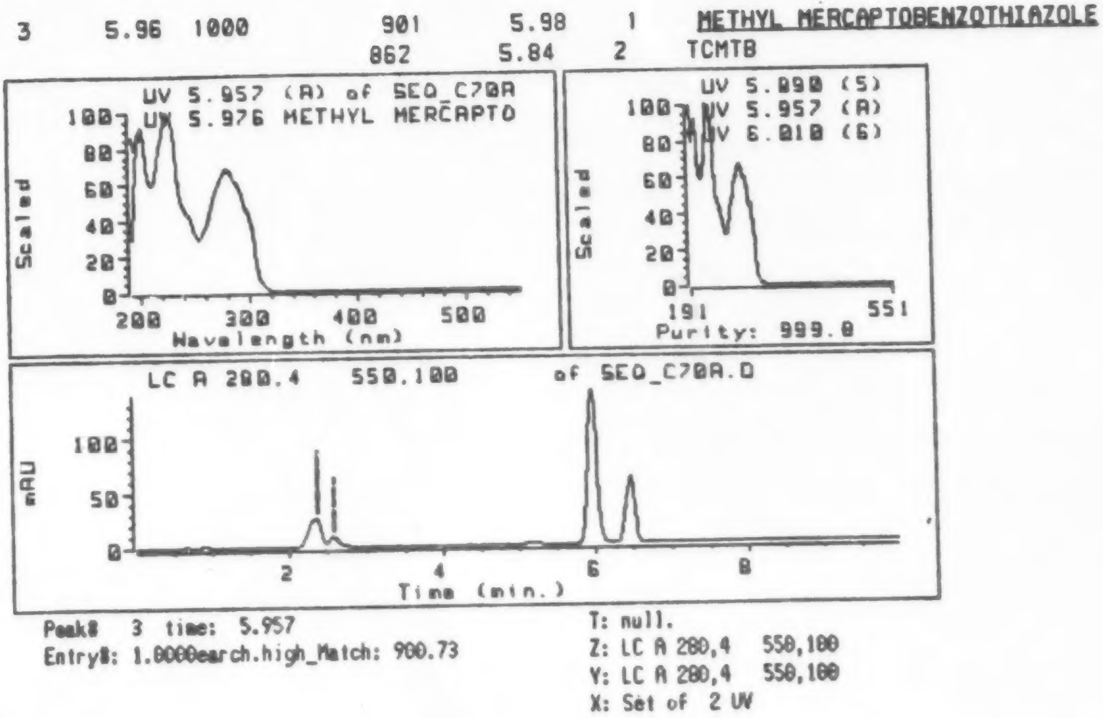
AUTOMATED LIBRARY SEARCH MACRO



```
3      5.96  1000          901       5.98     1     METHYL MERCAPTOBENZOTHIAZOLE
                           862       5.84     2     TCMTB
```

```
Peak#   3  time:  5.957                        T: null.
Entry#: 1.0000earch.high_Match: 900.73         Z: LC A 280,4    550,100
                                               Y: LC A 280,4    550,100
                                               X: Set of  2 UV
```

2-Methylmercaptobenzothiazole

Environment Canada   Environnement Canada

Canada

APPENDIX V

Environment Canada method for Cu-8 analysis

Environment Canada
Conservation and Protection

Laboratories
Pacific & Yukon Region

8-Hydroxy Quinoline V1.2 March, 1990 8HQ

High Performance Liquid Chromatography

SCOPE AND APPLICATION

This method is applicable to the determination of 8-Hydroxy Quinoline (8-HQ) in effluents, treated materials and sediments. The 8-HQ detection limit is 10 ug/l in effluents and sediment detection limits are currently under evaluation. The concentration of Bis-(8-Quinolinato) Copper ("Copper-8-Quinolinolate") is calculated from the 8-Hydroxy Quinoline concentration using a stoichiometric conversion factor of 1.212 based on the following equation:

$$1\ \mathrm{Cu} + 2\ \text{8-HQ} = 1\ \text{Bis-(8-Quinolinato) Copper}$$

SAMPLE HANDLING AND PRESERVATION

Note: All glassware, aluminum foil and sampling equipment that comes in contact with the sample must be acetone rinsed and heat-treated at 325 C for a minimum of 12 hr.

Samples should be collected in an all glass system and stored at 4 C or just above the freezing point. If Teflon lined caps are not available for the sampling containers then heat-treated aluminum foil must be used as a liner. At least 1 litre of sample should be collected.

SUMMARY OF METHOD

The sample is Methane Sulfonic Acid:Acetonitrile:Water extracted then cleaned up using solid phase extraction techniques (SPE). The sample is then analysed by High Performance Liquid Chromatography (HPLC).

METHOD PERFORMANCE

1. Four sample replicates yielded a 3.8 Average % Standard Deviation.
2. DI water solutions spiked with Nytek GD (10% Cu-8) at different concentration levels (8 - 50 ng/ml) over 4 replicates gave an average recovery of **89.7%** with a Standard Deviation of 4.3.
3. The linear coefficient of correlation was 1.000 over a 2 - 65 ng 8-HQ injection solution range, based on a 5 ul HPLC injection.

Compound	Calculated value (ng)	Found value (ng)	% Recovery
8-Hydroxy Quinoline	8.0	7.1	88.8
	10.0	8.6	86.0
	50.0	47.2	94.4

contd. ...

INTERFERENCES

1. Interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in HPLC chromatograms. All materials must be routinely demonstrated to be free from interferences under the conditions of analysis.
2. Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled.
3. Store standards at 4 C and protect from light. Stock standards should be checked frequently for signs of degradation.
4. Compounds co-eluting with 8-HQ and UV active at 250 nm are potential sources of interference.

SAFETY

Cu-8 and 8-HQ should be treated as a potential health hazard. Exposure should be kept at the lowest level possible. Use well vented fume hoods and wear protective clothes and gloves at all times when using these chemicals.

APPARATUS

All glassware must be washed with heavy-duty soap, rinsed with distilled water, rinsed with acetone and then heat-treated at 325 C for at least 12 hours prior to use. The glassware must be stored in an area protected from chemical contamination.

1. Graduated cylinders, 250-ml.
2. Volumetric flasks, 2-ml, 25-ml, 100-ml.
3. High performance liquid chromatograph (Hewlett-Packard 1090 equipped with a diode-array detector)
4. HPLC column (Hewlett Packard, ODS-HYPERSIL(C18), 5 um, 200 mm x 2.1 mm i.d.
5. Autosampler vials and caps, 2 ml.
6. Visiprep Solid Phase Extraction Vacuum Manifold, Supelco #5-7030.
7. C-18 columns, Supelclean Solid Phase, Supelco #5-7055.
8. Ultrasonic bath.
9. Shaker tubes, 100-ml, heavy-walled.
10. Buchner funnel, 5.0 cm.
11. Vacuum adaptor, 24/40.
12. GF/C filter paper, 4.5 cm.
13. Burrel wrist-action shaker.
14. Boiling flasks, 500-ml.

contd. ...

REAGENTS

1. Acetonitrile, Burdick & Jackson HPLC grade, filtered through Nylon 66 membrane filter (0.2 micron x 47 mm diameter).
2. Deionized water, filtered as above.
3. 8-Hydroxy Quinoline, 99+% purity from Aldrich #16,498-4.
4. 1-Octane Sulfonic Acid, 98% purity from Aldrich #22,156-2.
5. Methane Sulfonic Acid, 99% purity from Aldrich #M860-6.
6. 8-Hydroxy Quinoline Stock solution - weigh out 0.050 g into a 100-ml volumetric flask. The 8-HQ is dissolved by adding 1 ml of methane sulfonic acid, 10 ml of acetonitrile then diluting to 100.0 ml with water.
7. Extraction Solvent - add 10 ml of methane sulfonic acid and 100 ml of acetonitrile to a 1000-ml beaker and dilute to 1000 ml with water and mix for ten minutes.
8. HPLC Mobile Phase - add 2.6 ml of methane sulfonic acid and 2 g of octane sulfonic acid into a 1000-ml beaker, add 180 ml of acetonitrile and dilute to 1000 ml with DI water. Adjust the pH to 2.0 by addition of 1 N NaOH (approximately 30 ml).

PROCEDURE - GENERAL

1. Blanks are required for each new batch of reagents.
2. Duplicates and spiked samples must be run on a routine basis.
3. Standards should be injected frequently as a check on the stability of operating conditions.

PROCEDURE - EXTRACTION OF FORMULATIONS AND EFFLUENTS

1. Weigh out 0.100 g (formulations) or 100 ml (effluents) of well shaken sample into a 250-ml beaker.
2. Add 10 ml DI water and then dissolve by adding 50 ml Extraction Solvent. Add 1 ml methane sulfonic acid and place the beaker in a ultrasonic bath for 10 minutes.
3. During this time the C-18 columns are conditioned by first washing with 2 columns of acetonitrile and then 1 column of DI water.
4. The sample is transferred to the preconditioned C-18 column, allowed to pass through, eluted with 3 times 5 ml Extraction Solvent into a 25-ml volumetric flask and volumized with Extraction Solvent.
5. The sample is now ready for HPLC analysis. Dilution may be necessary.

PROCEDURE - EXTRACTION OF TREATED MATERIALS AND SEDIMENTS

1. Air dry approximately 15 g wet sample overnight. Weigh 10 g dried sample into a 100-ml heavy-walled shaker tube, add 50 ml Extraction Solvent and shake the sample on Burrel wrist action shaker for 2 hours.

contd. ...

PROCEDURE - EXTRACTION OF TREATED MATERIALS AND SEDIMENTS (ctd.)

2. Vacuum filter the Extraction Solvent extract through a Buchner funnel with a vacuum adapter. Use Whatman #1 filter paper and collect the filtrate in a 500-ml round bottom flask. Rinse the shaker tube twice, each with 20 ml of Extraction Solvent.
3. Using a minimum volume of Extraction Solvent quantitatively transfer the residue to a 100-ml volumetric flask using the Supelco filter tubes and the Visiprep filter apparatus. Volumize with Extraction Solvent.
4. The sample is now ready for HPLC analysis.

PROCEDURE - HPLC START-UP

NOTE: All control of the HPLC should be from the H/P 300 computer.

1. Ensure the HPLC 1090 mainframe is turned on then turn on the computer.
2. Check the solvent and distilled water reservoirs. Use freshly deionized water daily. Turn on Helium for solvent degassing.
3. Turn on the circulating bath for cooling of the autosampler.
4. Empty the waste solvent reservoir daily.
5. Check for sufficient printer paper.
6. Once computer has been booted it will show that you are at TOP LEVEL. Press the (F2) soft key, <DATA ACQUIS>. To move to different areas on the screen use the <TAB> key.

PROCEDURE - SET UP OF HPLC SEPARATION PARAMETERS

1. Once at <DATA ACQUIS> level press (F6) soft key, <EDIT PARAMS>. To get the Cu-8 conditions press (F1), <LOAD PARAMS>, press <?><RETURN>, and (F1), <SELECT ITEM> CU-8.A. This loads all the liquid chromatograph and diode array conditions required to identify 8-Hydroxy Quinoline. Press (F8), <EXIT>.
2. Press (F7), <MORE KEYS>, press (F3), <PUMP ON>, press (F7), <MORE KEYS>, press (F3), <MONITOR>. The wavelength screen will have spurious peaks and has to be balanced by pressing (F6), <BALANCE> key and should get a flat baseline. Do not proceed until this is correct. Press (F8), <EXIT> and then (F8), <QUIT>.
3. Once at TOP LEVEL again, press the (F5) soft key, <SEQUENCE>. The cursor key will be at the sequence table line, press <?><RETURN> and (F1), <SELECT ITEM> CU-8.R. Tab down to Sample Table file name, press <?><RETURN> and (F1), <SELECT ITEM> CU-8.S. Tab down to Data file name and set your Data file as follows: If running multiple samples and injections the file name is replaced with _____00A. The first 5 characters you can select as the descriptor. The last 3 characters (00A) are used by the chem station as a counter for the vial and injection numbers.

contd. ...

3.(ctd.)
ex. _____00A.D - Vial #0 (00) injection number 1(A).
_____02A.D _ Vial #2 (02) injection number 1(A).

Use an "A" for the fifth character for the Sequence run for automated runs. For routine samples the last four characters of the Laboratory Sample Number should be used:

0833AxxA.D

The counter sequence will identify the sub-sample.
When running an unattended analysis on the HPLC start at Sequence Line A.
Tab down to operator name and write in the operators name.

4. Press (F1) soft key <EDIT SEQ FILE>, then <?><RETURN> and (F1), <SELECT ITEM> CU-8.M. Set the range with how many samples are running and where they are situated in the auto-sampler (i.e. RANGE 00:30 REP 1 represents sample vial 1 to sample vial 30 with one injection). If any existing sequence files are present they can deleted by using the space bar and erasing the existing files. Press (F8), <EXIT> from Seq file.
5. Press (F2) soft key, <EDIT SAMPL TBL>. This table identifies every sample and how it is going to be analyzed (i.e. either a sample or calibration standard). Start by indexing the sample number, sample identification and then pressing (F8), <MORE INFO> to give the sample more identification. In this section you put in any dilution factor incorporated in the sample, is it a calibration sample or not. If it is a calibration standard at what level is it at with your existing calibration curve. If it is a recalibration standard you can either replace the level or average the level. Do this for every sample wanted to be analyzed. As in the Sequence file any existing samples in the table can be deleted by "space barring" through the Sample table and erasing existing samples.
6. Press (F8), <EXIT>, then press (F6), <CHECK METHODS>. If all is correctly set up you can press (F4), <START SEQUENCE> and unattended run can proceed.
7. Once START SEQUENCE has commenced press (F7), <HOLD METHOD> and check that the DATA ACQUIS FILE and all the conditions are correct. Check that the right vial is being injected. If the conditions are not met press (F8), <STOP METHOD>, wait for the first run to finish and recheck all parameters.
8. Note: You can check the integrity of your standard curve by re-running standards and "addressing" them in the sample table (see 5.).
9. Press (F8), <RESUME> once everything has been checked and is correct and run the samples.
10. The REPORT GENERATOR will provide all the quantitation results and report if the sample was a recalibration sample.

contd. ...

PROCEDURE -HPLC ANALYSIS

1. Liquid Chromatograph Conditions:

FLOW RATE: 0.50 ml/min.
SOLVENT A: 50% Cu-8 Mobil Phase
SOLVENT C1: 50% Acetonitrile
OVEN TEMP: 50.0 C

STOP TIME: 6.0 min
POST TIME: 3.0 min
INJ. VOL.: 10.0 ul

2. Diode Array Detector Conditions:

SIGNALS: A
SAMPLE: Wavelength = 250nm
Bandwidth = 4nm

REFERENCE: Wavelength = 550nm
Bandwidth = 100nm

Store the spectrum under peak controlled conditions with a threshold of 0.4 mAU, and a peakwidth of 0.100 min.

3. Inject exactly 5.0 ul of the 8-HQ Standard Mixes C1 - C5 and collect data. The individual pure standards may be made up in the appropriate concentrations and injected on the HPLC for confirmation of individual peak identification by retention time and full diode array library match.

COMPOUND	Concentration (ng/ul)				
	C1	C2	C3	C4	C5
8-HQ	64.3	32.3	12.9	6.4	1.5

4. Inject exactly 10.0 ul of each sample and collect data.

CALCULATIONS AND DATA PROCESSING

Note:
- For routine analysis the results reported and entered in the lab database will be for 8-Hydroxy Quinoline (8-HQ).
- For LEGAL analysis the results reported in the statement of analysis will be for Bis-(8-Quinolinato) Copper ("Copper-8-Quinolinolate") but the results entered in the laboratory database will be for 8-Hydroxy Quinoline (8-HQ), the same as for routine samples.

1. Calculation of 8-Hydroxy Quinoline (8-HQ) concentration.

[SAMPLE CONC.] = {[INJECTION SOLUTION CONC.] X [A] X [B] X [C]}

INJECTION SOLUTION CONC. = from Quantitation Report, in ng/ul
A = Final Volume of Injection Solution, in ml.
B = Dilution Factors (if any).

contd. ...

1. Calculation of 8-Hydroxy Quinoline (8-HQ) concentration.(ctd.)

C = for Effluent Samples = [1000]/[Volume Sample extracted - in ml]
for Treated Samples and Sediments = [1]/[Sample weight - in g]

Reporting Units: for Effluent Samples = ug/l
: for Sediments and Treated Samples = ug/g

2. Calculation of Bis-(8-Quinolinato) Copper ("Copper-8-Quinolinolate") concentration.

[BIS-(8-QUINOLINATO) COPPER CONC.] = A x 1.212

A = Concentration of 8-Hydroxy Quinoline (from Section 1.).
1.212 = The stoichiometric conversion factor of 8-Hydroxy Quinoline/ Bis-(8-Quinolinato) Copper (from the following equation).

1 Cu	+	2 8-HQ	=	1 Bis-(8-Quinolinato) Copper
(63.546 g/mole)		(145.16 g/mole)		(351.85 g/mole)

3. The concentration of the 8-Hydroxy Quinoline is entered in the laboratory database using "RENT" software and entering "8-HQ" for the Results (Billing) Code. The following statement will be added automatically to the report by the "NOTES" program:

"The concentration of Bis-(8-Quinolinato) Copper ("Copper-8-Quinolinolate") can be calculated by multiplying the 8-Hydroxy Quinoline (8-HQ) concentration by a stoichiometric conversion factor of 1.212."

4. For LEGAL analysis the following statement should be added to the statement of analysis:

"The concentration of Bis-(8-Quinolinato) Copper ("Copper-8-Quinolinolate") has been calculated by multiplying the 8-Hydroxy Quinoline (8-HQ) concentration by a stoichiometric conversion factor of 1.212 based on the following equation:

1 "Copper" + 2 "8-HQ" = 1 "Bis-(8-Quinolinato) Copper""

REFERENCES

[1] Hewlett Packard HP1090 Liquid Chromatograph Fact File Handbook, First Edition - November 1987.
[2] Analysis of 8-Hydroxy Quinoline Content of Nytek Formulations, Use Solutions and Treated Products (communication), Maag Agrochemicals, HLR Sciences, Inc., Vero Beach, Florida, 88/07/21.

contd. ...

REVISION HISTORY

Version 1.2	MAR/90	Change to microbore HPLC column for better sensitivity.
1.1	DEC/89	Documentation upgrade, addition of legal analysis qualifications.
1.0	MAR/89	New method.

contd. ...

PROGRAM USED BY HPLC COMPUTER SYSTEM (H/P 300 CHEMSTATION) TO IDENTIFY PEAKS FOR PURITY AND LIBRARY MATCH BASED ON SPECTRAL LIBRARY FIT AND RETENTION TIME

```
!------------------------------------------------------------------!
!   Macro for automated spectral library search                    !
!                                                                  !
!     Signal with peaks in z-register.                             !
!                                                                  !
!   AUTOLIB.G Version 4.01                                         !
!      First release.                                              !
!   AUTOLIB.G Version 4.06                                         !
!     If sequence running then no query for input and default     !
!       settings (library is taken from method data file param)    !
!------------------------------------------------------------------!

NAME AUTOLIB
  seqinf                ! sequence info
  CHR ,81
  EX X,Z
  gets header,z
  If Nobjects=0 Then
    Convert A$,"No signals in Z-register, please select first"
    lib_ABORT                  ! return to data editor macros
    RETURN
  EndIf
  Strpos "AUTOLIB 4.06 Initialized",AutolibIn$
  if len1=len2 then
    if value=1 then
    else
       INITAULIB     ! first time so initialize variables
  endif
else
       INITAULIB     ! first time so initialize variables
  format merged
  clear
  cl x
  cl y
  cl t

  if seq_run=0 then    ! no method or sequence so ask
    Convert A$,"N"
    Getvar A$,"Change settings (No)"
    strpos "Y",A$
    if value>0 then
```

contd. ...

```
       SETVAR          ! change settings
    endif
    DADlib FILE
  else                 ! sequence/method running
    DADlib FILE,,NOQ   ! open default library
  endif
  ex x,z
  ent
  mul 1
  DRAW sigtowin,y
  filestart=xlow
  fileend=xhigh
  ref_time=filestart
  spec filend
  lastspec=scan_num    ! get last spectrum available
  gets header,z
! dychar=charsize/10*(yhigh-ylow)/40
  dychar=charsize/10*(yhigh-ylow)/(40-fieldsize-7)  !???????????
  ysc=yhigh+5*dychar
  if numpeaks=0 then
    INTEGRATE Z
    numpeaks=npeaks
  endif
  PeaksFound=NumPeaks
  If PeaksFound=0 Then
  Convert A$,"No peaks found; please change int params first"
    lib_ABORT
    RETURN
  EndIf

  if 1=2 then
    size=0
    x1=0.5
    while size<20
      annot sigtowin,0,size,0,x1,0.3,"C&P Library search"
      size=size+1
      x1=x1-0.01125
      annot sigtowin,0,size,3,x1,0.3,"C&P Library search"
    endwhile
  endif

  if doprint=1 then
     PRHEAD
  endif
```

contd. ...

```
CurPeak=0
dr sigtowin,y
format merged
while CurPeak<Peaksfound
  CurPeak=CurPeak+1
  MESS "Peak spectra search....                        ",,0,-5
  PEAKS CurPeak,,none,Z,1,st_wave:en_wave
  Crest=Ret_Time
  rol -1
  spec ret_time,st_wave:en_wave
  top_time=ret_time
  if (ret_time-start_time)*(end_time-ret_time)>=0 then

    Convert A$,"Peak#",CurPeak:4:0,"  time:",RET_TIME:7:3
    mes A$,,0,-6

   !--  get the spectr/a/um  --!
    if dopeakpur=1 then
      PEAK_SPEC
    else
      if dosubref=1 then
        if ref_spec<0 then
           PEAK_SPEC
        endif
      endif
    endif

    !-- subtract reference spectrum  --!
    if dosubref>0 then
       SUBREF
    endif

    !-- remove empty objects  --!
    gets header,x
    objs=nobjects
    i=1
    while i<=objs
      gets header,x,i
      if ylow=0 then
        if yhigh=0 then
          extr i
          rol -1
          i=i-1
```

contd. ...

```
     objs=objs-1
    endif
  endif
  i=i+1
endwhile

gets header,x
if nobjects>0 then

  if nobjects=2 then
    if dopeakpur=0 then
      extr
      add
      mul 0.5
   endif
  endif
   SMDERNORM
  gets header,x
  if nobjects>1 then
    dr purtowin,x
    compare ab_thres,0,x,1,x,nobjects
    Convert A$,"Purity:",match:6:1
    ann purtowin,,,,,,A$,xaxis
    dr purtowin,x
    peak_pur=match
    if match>purthres then
      if dosubref>0 then
        if ref_spec<0 then
          extr
          add
          mul 0.5
        else
          ro -1
          spec top_time,st_wave:en_wave
       SUBREF
        endif
      else
        ro -1
        spec top_time,st_wave:en_wave
      endif
       SMDERNORM
    else
```

contd. ...

```
     DADLib SEARCH,SPECTRUM,ab_thres,cor_sh,leftwin:rightwin,x,nobjects
   endif
 else
   peak_pur=-1000
 endif
 DADLib SEARCH,SPECTRUM,ab_thres,cor_sh,leftwin:rightwin,x,1
 if doprint=1 then
   write 701,CurPeak:2:0,crest:9:2
   if dopeakpur=1 then
      if peak_pur>-0.1 then
        writereal 701,peak_pur:6:0
     else
       write 701,"   ???"
     endif
  else
     write 701,"   ---"
   endif

   upslope=0
   downslope=0
   DADlib RES,1,x,1
   if entry>0 then
     n_object=1
      gets header,x
     if nobjects>1 then
       upslope=1
     endif
      PRSEAR
     upslope=0
   else
     writeln 701,match:12:0
     endif
     gets header,x
     if nobjects>1 then
       n_object=nobjects
     downslope=1
      PRSEAR
     downslope=0
  endif

  mathfunc trunc,CurPeak/5,x
  if CurPeak-(x*5)=0 then
    writeln 701,""
  endif
endif
```

contd. ...

```
gets header
if nobjects=1 then
  convert A$,""
  DADlib RES,1,x,1
  high_match=match
else
  if peak_pur>purthres then
    convert A$,""
  else
    convert A$,"*"
  endif
  high_match=0
  DADlib RES,1,x,1
  If entry>0 then
    high_match=match
  endif
  DADlib RES,1,x,nobjects
  If entry>0 then
    if Match>high_match then
            high_match=match
  else
  DADlib RES,1,x,1
  high_match=match
    endif
  endif
endif
If entry>0 then
  if high_Match>=searthres then
    DADlib READ,entry,st_wave:en_wave
    convert A$,A$,Comp_name$
    norm 100
    if cor_sh=0 then
    else
      gets header
      trans xlow:xhigh,(xlow+cor_sh):(xhigh+cor_sh)
    endif
    merge

    dr spetowin
    SCREENPRINT 640,500
    mess "Entry#:",entry,0,-5
    mess "high_Match:",Match,20,-5
  else
```

contd. ...

```
          convert A$,A$,"?"
        endif
      else
        convert A$,A$,"?"
      endif
      if adjanot=1 then
        gets header,y
        gets point,start_time:end_time,z
        ymax=y+0.5*dychar
      endif
      if ymax+((fieldsize+5)*dychar)>ysc then
        ysc = ymax+((fieldsize+5)*dychar)
      endif
      AN sigtowin,anotrot,charsize,3,crest,ymax,A$[1:fieldsize],DATA,1
    endif
  endif
endwhile
Convert A$,"Library : ",lib_name$
AN sigtowin,,,6,0.15,0.90,A$
AN sigtowin,,,6,0.15,0.94,"Results of spectral library search"
Convert A$,"Match > ",searthres:3:0
an sigtowin,0,10,3,0.02,0.01,A$
clear
gets header,y
dr 3,y,,ylow:ysc*0.8
if doprint=1 then
  write   701,"----------------------------------"
  writeln 701,"----------------------------------"
  writeln 701,""
  makeprint
endif
CL
CHR ,81
EX X,Z
EV CU8.E
START SET
INT Z
DRAW 3,Z
GETS Z
DRAW 3,Z,,0:YHIGH*1.2
PAGES 1
DRAW PRINTER:,Z,,0:YHIGH*1.2
QUIT
RETURN
```

contd. ...

```
NAME MAKEPRINT
  win 4,0:1,0:1
  clear
  gets header,y
  dr 4,y,,ylow:ysc
  screenprint 640,500

  Clear
  dr 3,y,,ylow:ysc
  return
NAME MAKEPLOT
  win 4,0:1,0:1
  clear
  gets header,y
  dr 4,y,,ylow:ysc
  redraw 705

  Clear
  dr 3,y,,ylow:ysc
  return
! ------------------------------------!
!                                     !
!-------------------------------------!
NAME CHECKWAVE
  scan 1
  rol -1
  gets header,t,1
  if en_wave<=st_wave then
    st_wave=xlow
    en_wave=xhigh
  else
    if st_wave<xlow then
      st_wave=xlow
    endif
    if en_wave> >xhigh then
      en_wave=xhigh
    endif
    if en_wave<=st_wave then
      st_wave=xlow
      en_wave=xhigh
    endif
  endif
  RETURN
```

contd. ...

```
!-----------------------------------!
! change settings                   !
!-----------------------------------!

NAME SETVAR
  if leftwin>-0.01 then
    Convert A$,"Yes"
  else
    Convert A$,"No"
  endif
  Convert B$,"Library time search window (",A$,") >"
  getvar A$,B$
  strpos "Y",A$
  if value>0 then
    if leftwin<0 then
      leftwin=5
    endif
    Convert A$,"Search window (% of ret. time) (",LeftWin:0:1,") >"
    Getvar LeftWin,A$
    rightwin=leftwin
  else
    leftwin=-1
    rightwin=-1
  endif
  if dosubref=1 then
    Convert A$,"Yes"
  else
    Convert A$,"No"
  endif
  Convert B$,"Subtract background (",A$,") >"
  getvar A$,B$
  strpos "Y",A$
  if  value>0 then
    dosubref=1
    value=-1
    while value<0
      if ref_spec=0 then
        Convert A$,"Base"
      else
        if ref_spec<0 then
          Convert A$,"Apex"
        else
          Convert A$,"Ref"
```

contd. ...

```
      endif
    endif
    Convert B$,"Type of background, Ref/Base/Apex (",A$,") >"
    getvar A$,B$
    strpos "BA",A$
    if value>0 then
      ref_spec=0
    else
      strpos "AP",A$
      if value>0 then
        ref_spec=-1
      else
        strpos "RE",A$
        if value>0 then
          if ref_spec<0 then
            ref_spec=0.01
          endif
          convert A$,"Give time of reference spectrum (",ref_spec:7:3,") >"
          Getvar ref_spec,A$
        else
          mess "unknown type"
        endif
      endif
    endif
  endwhile
else
  dosubref=-1
endif
 CHECKWAVE
Convert A$,"Wavelength range from (",st_wave:0:1,") >"
Getvar st_wave,A$
Convert A$,"Wavelength range to (",en_wave:0:1,") >"
Getvar en_wave,A$
if do smooth=1 then
  Convert A$,"Yes"
else
  Convert A$,"No"
endif
Convert B$,"Smooth before search (",A$,") >"
getvar A$,B$
strpos "Y",A$
if  value>0 then
```

contd. ...

```
  dosmooth=1
  Convert A$,"Number of smooth points, >5 (",smoothfac:0:0,") >"
  Getvar smoothfac,A$
else
  do smooth=0
endif
Convert A$,"Match threshold for library (",SearThres:0:0,") >"
Getvar SearThres,A$
if dopeakpur=1 then
  Convert A$,"Yes"
else
  Convert A$,"No"
endif
Convert B$,"Peak purity check (",A$,") >"
getvar A$,B$
strpos "Y",A$
if  value>0 then
  dopeakpur=1
  Convert A$,"Match threshold for purity (",purThres:0:0,") >"
  getvar purthres,A$
else
  dopeakpur=0
endif
if doprint=1 then
  Convert A$,"Yes"
else
  Convert A$,"No"
endif
Convert B$,"Printout of results (",A$,") >"
getvar A$,B$
strpos "Y",A$
if value>0 then
  doprint=1
else
  doprint=0
endif
RETURN
```

contd. ...

```
!---------------------------------------!
! print heading                         !
!---------------------------------------!
NAME PRHEAD
    tof
    writeln 701,""
    write   701,"
    writeln 701,"C&P automated Library search macro (rev 4.06)#27&d@#15  ------"
!   writeln 701,"#10"
!   screenprint 640,500,printer:
    writeln 701,"#10"
    writeln 701,"Library file          : ",FILE_L$
    writeln 701,"        name          : ",lib_name$,#10
    writeln 701,"Match threshold       : ",Searthres:0:0
    if cor_sh>0 then
      writeln 701,"WL calibration cor.   : ",cor_sh:0:1," nm"
    endif
    write 701,"reference spectrum    : "
    if dosubref=1 then
      if ref_spec= then
        writeln 701, "Baseline"
      else
        if ref_spec<0 then
          writeln 701,"Apex"
        else
          writeln 701,ref_spec:0:2," min"
        endif
      endif
    else
      writeln 701,"None"
    endif
    if der_order>0 then
      writeln 701,"Order of derivative   : ",der_order:0:0
    endif
    write 701,"Smooth                : "
    if dosmooth=1 then
      writerealn 701,smoothfac:0:0
    else
      writeln 701,"None"
    endif
     CHECKWAVE
    writeln 701,"Wave range            : ",st_wave:0:1," to ",en_wave:0:1," nm"
    write 701,"Time window           : "
    if leftwin<0 then
```

contd. ...

```
      write 701,"None"
    else
      writeReal 701,leftwin:0:1
    endif
    if leftwin=rightwin then
    else
      write 701," to "
      if rightwin<0 then
        write 701,"None"
      else
        writeReal 701,rightwin:0:1
      endif
     endif
     writeln 701," %"
!   writeln 701,"Absorbance threshold  : ",ab_thres:0:1," %"
    write 701,"Peak purity threshold : "
    if dopeakpur=1 then
      writeRealn 701,purthres:0:0
    else
      writeln 701,"none"
    endif

  writeln 701,"#10#10File    : ",file_d$
  writeln 701,"Date    : ",inj_date$
  writeln 701,"Name    : ",data_name$
  writeln 701,"Info    : ",misc_info$
  writeln 701,"Operator: ",operator$
  if inj_num>0 then
    writeln 701,""
    write   701,"Sequence index : "seq_index:0:0
    writeln 701,"  vial : ",bottle:0:0," replicate : ",inj_num:0:0
  endif

  write   701,"#10#10----- Sample ------     #27&dD#14Match#27&d@#15"
  write   701,"     -----------------"
  writeln 701," Library ---------------------"
  writeln 701,"Peak#----------------------------------------------"
  writeln 701,"----------------------------"
  RETURN !PRHEAD!
```

contd. ...

```
!------------------------------------!
! ads a spectrum to x register       !
!------------------------------------!
NAME AD_SPEC
  if ret_time<start_time then
    clear x
  else
    if ret_time>end_time then
      clear x
    endif
  endif
  gets header
  if nobjects=0 then
    ro -1
  else
    Convert Z$,header$
    gets header,y
    if nobjects=0 then
      ro -1
       ex x,t
    else
      i=1
      while i<=nobjects
        gets header,y,i
        strpos header$,Z$
        if value=1 then
          if len1=len2 then
            value=0
            i=nobjects
          else
            value=1
          endif
        else
          value=1
        endif
        i=i+1
      endwhile
      if value=0 then
        ro -1
      else
        merge
      endif
    endif
 endif
 RETURN
```

contd. ...

```
!-----------------------------------!
! get up/top/down spectra           !
!-----------------------------------!
NAME PEAK_SPEC
  cl x
  Spec Crest-(Peak_Width)/2,st_wave:en_wave
   AD_SPEC
  Spec Crest,st_wave:en_wave
   AD_SPEC
  Spec Crest+(Peak_Width)/2,st_wave:en_wave
   AD_SPEC
  RETURN !PEAK_SPEC!

!-----------------------------------!
! find baseline spectrum.           !
!-----------------------------------!
NAME GETBSPEC
  spec crest,st_wave:en_wave
  found=0
  while found=0
    if value=69 then
      ref_time=ret_time
      found=1
    else
      rol -1
      if scan_num>=lastspec then
        mes "No base spectrum found, set to:",ref_time,0,-5
        spec ref_time,st_wave:en_wave
        found=1
      else
        scan scan_num+1,st_wave:en_wave
      endif
    endif
  endwhile
  RETURN !GETBSPEC!
```

contd. ...

```
!-------------------------------------!
! subtract ref spec                   !
!-------------------------------------!
NAME SUBREF
  if ref_spec=0 then
     GETBSPEC
  else
    if ref_spec<0 then
      mul -1
      Spec Crest,st_wave:en_wave
      mul -1
    else
      Spec ref_spec,st_wave:en_wave
      ref_time=ret_time
    endif
  endif
  subtr
  SCREENPRINT 640,500

  RETURN !SUBREF!

!-------------------------------------!
! subtract ref spec                   !
!-------------------------------------!
NAME PRSEAR
  DADlib RES,1,x,n_object
  if entry>0 then
    il=1
    doit=1
    while doit>0
      DADlib RES,il,x,n_object
      doit=0
      if entry>0 then
        DAdlib Read,entry
        rol -1
        if il=1 then
          doit=1
        endif
        if match>searthres then
          doit=1
        endif
      endif
      if doit>0 then
        if il=1
```

contd. ...

```
        gets header,x,n_object
        strpos "(",header$
        Convert time,header$[3:(value-1)]
        if upslope=1 then
          writeln 701,""
          write 701,time:11:2,"          up"
        else
          if downslope=1 then
            write 701,time:11:2,"        down"
          else
            write 701,"     "
          endif
        endif
      else
        write 701,"                      "
      endif
      write 701,match:07:0,Ret_time:10:2,entry:5:0

      convert A$,"      "
      if match>searthres then
        if il=1 then
          Convert comp_name$,"#27&dD#14",comp_name$,"#27&d@#15"
          if upslope=1 then
            convert A$,"  * "
          endif
          if downslope=1 then
            convert A$,"  * "
        endif
      endif
    endif
      writeln 701,A$,comp_name$
    endif
    il=il+1
  endwhile
endif
RETURN
```

contd. ...

```
!-------------------------------------!
!  get derivative/smooth/norm spectra  !
!-------------------------------------!
NAME SMDERNORM
  if der_order>0 then
    i1=1
    while i1<=der_order
      derivative
      i1=i1+1
    endwhile
  else
    if dosmooth=1 then
      smooth smoothfac
    endif
  endif
  norm 100
  RETURN !SMDERNORM!
!-------------------------------------!
! ABORT                               !
!-------------------------------------!
Name lib_ABORT
  mes A$,,0,-5
  if seq_run=yes then
    RETURN                    ! return to caller
  endif
                              ! stop

!-------------------------------------!
! initializes the macro               !
!-------------------------------------!
NAME INITAULIB
  dosubref=-1
  ref_spec=-1          ! no reference spectrum
  der_order=0          ! order of derivative
  dopeakpur=1          ! peak purity check (1=yes 0=no)
  adjanot=1            ! adjust compound nam
  smoothfac=11         ! smooth factor
  doprint=1            ! printout of results (1=yes, 0=no)
  fieldsize=15         ! annotation field size
  charsize=13          ! annotation character size
  anotrot=90           ! annotation string rotation
  sigtowin=1
  spetowin=2
  purtowin=4
```

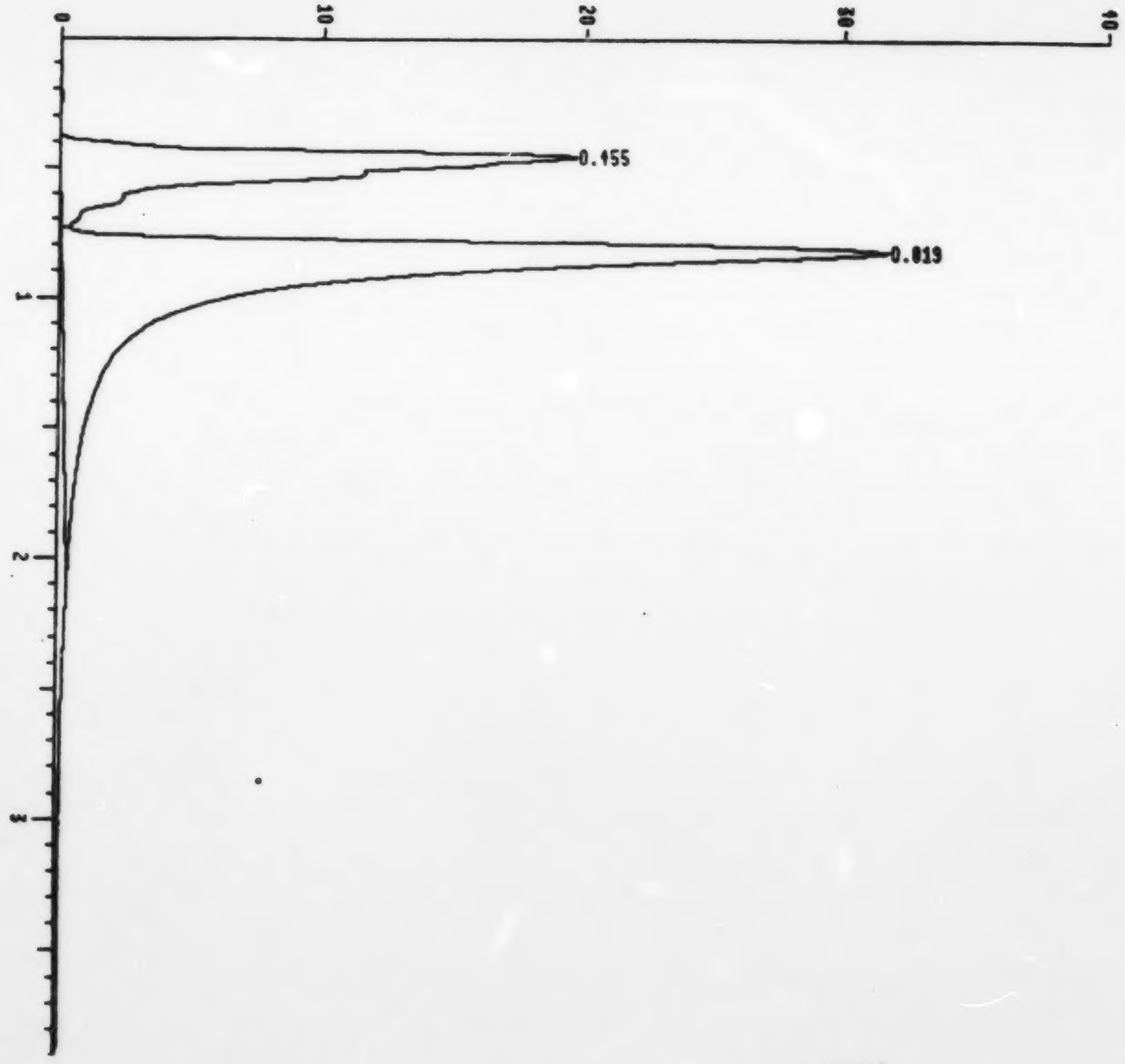
contd. ...

```
 seqinf
 if seq_run=0 then     ! Take the values from DATA EDITOR
   purthres=matchthres! purity threshold (above this threshold peak is pure)
   leftwin=leftwin     ! search window size 5 % of rete
   st_wave=startwave   ! start wavelength range
   en_wave=endwave     ! end wavelength range
   cor_sh=waveshift    ! wavelength calibration shift for spectral library
   ab_thres=absthres   ! mAU threshold for spectral library
   searthres=matchthres ! Match factor above which peak is identified.
 else                  ! set values
   purthres=500        ! purity threshold (above this threshold peak is pure)
   leftwin=20          ! search window size 5 % of retention time
   rightwin=15         ! when <0 then no search window
   st_wave=0           ! start wavelength range
   en_wave=0           ! end wavelength range
   cor_sh=0            ! wavelength calibration shift for spectral library
   ab_thres=2          ! mAU threshold  peak is identified.
   searthres=500       ! Match factor above which peak is identified.
 endif

 Convert AutolibIn$,"AUTOLIB 4.06 Initialized"  ! set autolib is initialized.

 RETURN

!-------------------------------------!
! removes the macro                   !
!-------------------------------------!
NAME REMUSERMAC
 RemAUTOLIB,MakePrint,MakePlot,InitAuLib,CheckWave,SetVar,PrHead,Ad_Spec
 Rem Peak_SPec,GetBspec,SubRef,PrSear,SmDerNorm,Lib_Abort
 RETURN

!-------------------------------------!
!                                     !
!-------------------------------------!
NAME DOUSERMAC
   VM
  OLD_VM=VM_MEM
  VM ,60000
   AUTOLIB
  VM ,OLD_VM
   DISPSTATUS=1
   REMUSERMAC
  RETURN
```

contd. ...

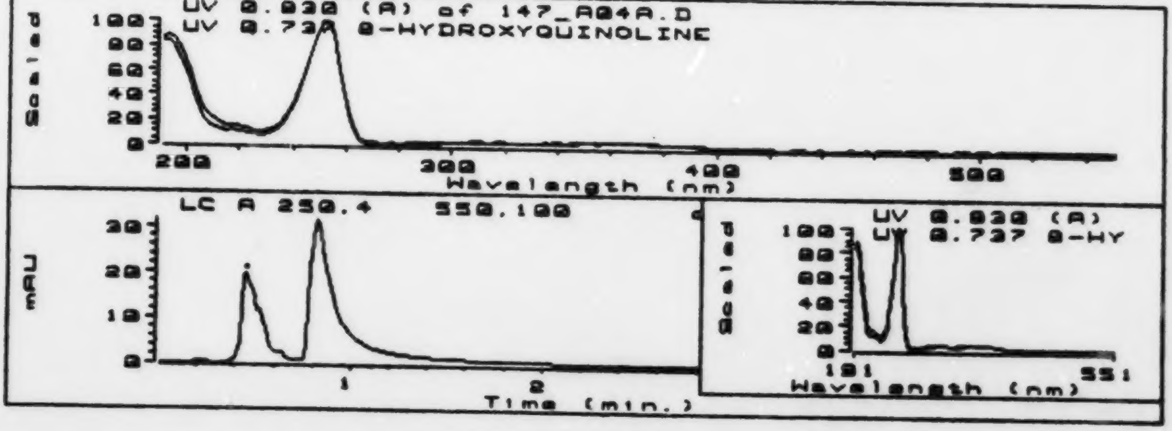
HPLC CHROMATOGRAM AND LIBRARY SEARCH RESULTS FROM A STANDARD OF 8-HYDROXYQUINOLINE:

1: LC A 250,4 550,100 of 147_A04A.D

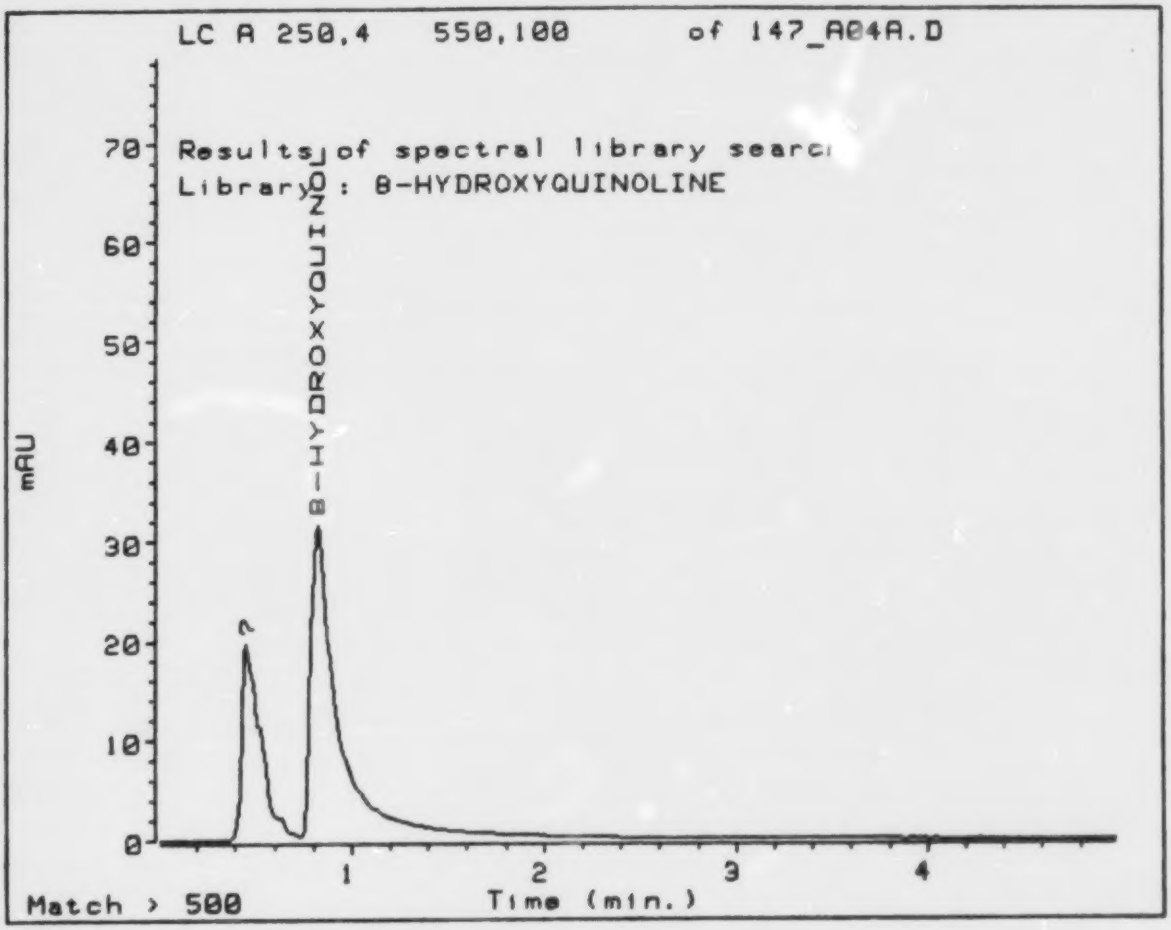


contd. ...

```
------ C&P automated Library search macro (rev 4.06) ------

Library file            : DATA:CU-8.L
        name            : 8-HYDROXYQUINOLINE

Match threshold         : 500
reference spectrum      : None
Smooth                  : None
Wave range              : 191.0 to 551.0 nm
Time window             : 20.0 to 15.0 %
Peak purity threshold   : 500

File     : DATA:147_A04A.D
Date     :  9 Feb 90   8:50 am
Name     :
Info     :
Operator:

Sequence index : 1  vial : 4  replicate : 1

----- Sample ------       Match    ---------------- Library ------------------
Peak#  Time  Purity                 Time   Entry#     Name
-------------------------------------------------------------------------------
 1     0.46   979            0
 2     0.82  1000          994      0.74     1     -HYDROXYQUINOLINE
```



contd. ...


LC A 250,4 550,100 of 147_A04A.D
Results of spectral library searc
Library : 8-HYDROXYQUINOLINE
8-HYDROXYQUINOL
?
mAU
70
60
50
40
30
20
10
0
1
2
3
4
Time (min.)
Match > 500

APPENDIX VI

Environment Canada method for IPBC analysis

Environment Canada
Conservation and Protection

Laboratories
Pacific & Yukon Region

3-Iodo-2-Propynylbutylcarbamate V1.0 August, 1989 IPBC

High Performance Liquid Chromatography

SCOPE AND APPLICATION

This method is applicable to the determination of 3-Iodo-2-Propynylbutylcarbamate (IPBC) in effluents and sediments. The IPBC detection limit is 20 ug/l in effluents. This translates into 260 ug/l NP-1, based on a formulation concentration of 7.65% IPBC. Sediment detection limits are currently under evaluation.

SAMPLE HANDLING AND PRESERVATION

Note: All glassware, aluminum foil and sampling equipment that comes in contact with the sample must be acetone rinsed and heat-treated at 325 C for a minimum of 12 hr.

Samples should be collected in an all glass system and stored at 4 C or just above the freezing point. If Teflon lined caps are not available for the sampling containers then heat-treated aluminum foil must be used as a liner. At least 1 litre of sample should be collected.

SUMMARY OF METHOD

The sample is extracted with methylene chloride and then the extract is filtered and evaporated to dryness. The residue is dissolved in methanol and the sample then analysed by High Performance Liquid Chromatography (HPLC).

METHOD PERFORMANCE

1. Four sample replicates yielded a 3.8 Average % Standard Deviation.
2. A DI water solution spiked with NP-1 at the following 600 ng/l level over 4 replicates gave an average recovery of 88.4% with a Standard Deviation of 3.8.

Compound	Calculated value (ng)	Found value (ng)	% Recovery
IPBC (in NP-1)	152	134	88.4%

3. The linear coefficient of correlation was 1.000 over a 3 - 155 ng IPBC Injection Solution range, based on 7.65% IPBC content of NP-1 and a 10 ul HPLC injection.

contd. ...

INTERFERENCES

1. Interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in HPLC chromatograms. All materials must be routinely demonstrated to be free from interferences under the conditions of analysis.
2. Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled.
3. Store standards at 4 C and protect from light. Stock standards should be checked frequently for signs of degradation.
4. Compounds co-eluting with IPBC and UV active at 254 nm are potential sources of interference.

SAFETY

IPBC should be treated as a potential health hazard. Exposure should be kept at the lowest level possible. Use well vented fume hoods and wear protective clothes and gloves at all times when using these chemicals.

APPARATUS

All glassware must be washed with heavy-duty soap, rinsed with distilled water, rinsed with acetone and then heat-treated at 325 C for at least 12 hours prior to use. The glassware must be stored in an area protected from chemical contamination.

1. Graduated cylinders, 250-ml.
2. Boiling flasks, 500-ml.
3. Separatory funnels, 500-ml.
4. Vials, 7-ml with foil cap liners.
5. Volumetric flasks, 2-ml, 10-ml.
6. Rotary evaporator.
7. Centrifuge.
8. Glass columns, 26 cm x 2.0 cm, with sintered glass frits and teflon stopcocks.
9. High performance liquid chromatograph (Hewlett-Packard 1090 equipped with a diode-array detector).
10. HPLC column (Hewlett Packard, ODS(C18)), 5 um, 100 mm x 4.6 mm i.d.
11. Autosampler vials and caps (2 ml).
12. Visiprep Solid Phase Extraction Vacuum Manifold, Supelco #5-7030.
13. Supelclean Solid Phase Filtration Tubes, Supelco #5-7026.
14. Buchner funnel, 5.0 cm.
15. Vacuum adapter, 24/40.
16. GF/C filter paper (4.5 cm).
17. Burrel wrist-action shaker.

contd. ...

REAGENTS

1. Methanol, Burdick & Jackson HPLC grade, filtered through Nylon 66 membrane filter (0.2 micron x 47 mm diameter).
2. Methylene chloride, Burdick & Jackson HPLC grade.
3. Acetonitrile, Burdick & Jackson HPLC grade, filtered through Nylon 66 membrane filter (0.2 micron x 47 mm diameter).
4. Deionized water, filtered through Nylon 66 membrane filter (0.2 micron x 47 mm diameter).
5. Troysan Polyphase 100, (IPBC), 99.6%, Troy Chemical Corporation, Newark, New Jersey.

PROCEDURE - GENERAL

1. Blanks are required for each new batch of reagents.
2. Duplicates and spiked samples must be run on a routine basis.
3. Standards should be injected frequently as a check on the stability of operating conditions.

PROCEDURE - EXTRACTION OF EFFLUENTS

1. Add 250 ml of the shaken sample to a 500-ml separatory funnel.
2. Extract the IPBC by adding 75 ml methylene chloride and vigorously shaking the separatory funnel for 2 minutes.
3. Drain the solvent fraction through a funnel plugged with glass wool and 5 g $Na2SO4$ into a 500-ml round bottom flask.
4. Repeat the extraction twice more using 75 ml methylene chloride each time.
5. Rotary evaporate the combined extracts to dryness.
6. Using a minimum volume of methanol, quantitatively transfer the residue to a 2-ml volumetric flask using the Supelco filter tubes and the Visiprep filter apparatus.
7. The sample is now ready for HPLC analysis.

PROCEDURE - EXTRACTION OF SEDIMENTS

1. Air dry approximately 15 g wet sample overnight. Weigh out 10 g of the air-dried sample into a 100-ml heavy-walled shaker tube and add 50 ml methylene chloride. Shake the sample on Burrel wrist action shaker for 2 hours.
2. Vacuum filter the methylene chloride extract through a Buchner funnel with a vacuum adapter. Use Whatman #1 filter paper and collect the filtrate in a 500-ml round bottom flask. Rinse the shaker tube twice, each with 25 ml of methylene chloride.
3. Add approximately 5 g heat treated sodium sulfate to the flask, cover and leave for about 30 min.

contd. ...

4. Transfer extract to another 500-ml round-bottom flask and rotary evaporate the extract to dryness.
5. Using a minimum volume of methanol, quantitatively transfer the residue to a 2-ml volumetric flask using the Supelco filter tubes and the Visiprep filter apparatus.
6. The sample is now ready for HPLC analysis.

ANALYSIS - PROCEDURE FOR HPLC START UP

NOTE: All control of the HPLC should be from the HP 300 computer.

1. Ensure the HP 1090 mainframe is turned on then turn on the computer.
2. Check the solvent and distilled water reservoirs. Use freshly deionized water daily. Turn on Helium for solvent degassing.
3. Turn on the circulating bath for cooling of the autosampler.
4. Empty the waste solvent reservoir daily.
5. Check for sufficient printer paper.
6. Once computer has been booted it will show that you are at TOP LEVEL. Press the (F2) soft key, <DATA ACQUIS>. To move to different areas on the screen use the <TAB> key.

ANALYSIS - PROCEDURE FOR SETTING SEPARATION PARAMETERS

1. Once at <DATA ACQUIS> level press (F6) soft key, <EDIT PARAMS>. To get the NP-1 conditions press (F1), <LOAD PARAMS>, press <?><RETURN>, and (F1), <SELECT ITEM> NP1.A. This loads all the liquid chromatograph and diode array conditions required to identify 3-Iodo-2-Propynylbutylcarbamate. Press (F8), <EXIT>.
2. Press (F7), <MORE KEYS>, press (F3), <PUMP ON>, press (F7), <MORE KEYS>, press (F3), <MONITOR>. The wavelength screen will have spurious peaks and has to be balanced by pressing (F6), <BALANCE> key and should get a flat baseline. Do not proceed until this is correct. Press (F8), <EXIT> and then (F8), <QUIT>.
3. Once at TOP LEVEL again, press the (F5) soft key, <SEQUENCE>. The cursor key will be at the sequence table line, press <?><RETURN> and (F1), <SELECT ITEM> NP1.R. Tab down to Sample Table file name, press <?><RETURN> and (F1), <SELECT ITEM> NP1.S. Tab down to Data file name and set your Data file as follows: If running multiple samples and injections the file name is replaced with _____00A. The first 5 characters you can select as the descriptor. The last 3 characters (00A) are used by the chem station as a counter for the vial and injection numbers.

ex. _____00A.D - Vial #0 (00) injection number 1(A).
_____02A.D _ Vial #2 (02) injection number 1(A).

contd. ...

3.(ctd.) Use an "A" for the fifth character for the Sequence run for automated runs. For routine samples the last four characters of the Laboratory Sample Number should be used (ex. 0833AxxA.D). The counter sequence will identify the sub-sample. When running an unattended analysis on the HPLC start at Sequence Line A then tab down to OPERATOR NAME and fill in as appropriate.

4. Press (F1) soft key <EDIT SEQ FILE>, then <?><RETURN> and (F1), <SELECT ITEM> NP1.M. Set the range with how many samples are running and where they are situated in the auto-sampler (i.e. RANGE 00:30 REP 1 represents sample vial 1 to sample vial 30 with one injection). If any existing sequence files are present they can deleted by using the space bar and erasing the existing files. Press (F8), <EXIT> from Seq file.
5. Press (F2) soft key, <EDIT SAMPL TBL>. This table identifies every sample and how it is going to be analyzed (i.e. either a sample or calibration standard). Start by indexing the sample number, sample identification and then pressing (F8), <MORE INFO> to give the sample more identification. In this section you put in any dilution factor incorporated in the sample, is it a calibration sample or not. If it is a calibration standard at what level is it at with your existing calibration curve. If it is a recalibration standard you can either replace the level or average the level. Do this for every sample wanted to be analyzed. As in the Sequence file any existing samples in the table can be deleted by "space barring" through the Sample table and erasing existing samples.
6. Press (F8), <EXIT>, then press (F6), <CHECK METHODS>. If all is correctly set up you can press (F4), <START SEQUENCE> and unattended run can proceed.
7. Once START SEQUENCE has commenced press (F7), <HOLD METHOD> and check that the DATA ACQUIS FILE and all the conditions are correct. Check that the right vial is being injected. If the conditions are not met press (F8), <STOP METHOD>, wait for the first run to finish and recheck all parameters.
8. Note: You can check the integrity of your standard curve by re-running standards and "addressing" them in the sample table (see 5.).
9. Press (F8), <RESUME> once everything has been checked and is correct and run the samples.
10. The REPORT GENERATOR will provide all the quantitation results and report if the sample was a recalibration sample.

PROCEDURE - HEWLETT-PACKARD 1090 HPLC SET UP CONDITIONS

1. Liquid Chromatograph Conditions:

FLOW:	2.00 ml/min.	POST TIME:	3.00 min
SOLVENT A:	60% H2O	STOP TIME:	10.0 min
SOLVENT C1:	40% METHANOL	INJECTION VOLUME:	10.0 ul
OVEN TEMP:	40.0 C		

GRADIENT CHANGE: TIME 0.0 min = 40% Methanol.
TIME 10.0 min = 100% Methanol.

contd. ...

2. Diode Array Detector Conditions:

SIGNALS: A
SAMPLE (nm) Wavelength : 254
Bandwidth : 4

REFERENCE (nm) Wavelength : 400
Bandwidth : 100

Store the spectrum under peak controlled conditions with a threshold of 0.4 mAU, and a peakwidth of 0.100 min.

3. Inject exactly 10.0 ul of the NP-1 Standard Mixes N1 - N5 and collect data. The individual pure standards may be made up in the appropriate concentrations and injected on the HPLC for confirmation of individual peak identification by retention time and full diode array library match.

COMPOUND	CONCENTRATION (ng/ul)				
	N1	N2	N3	N4	N5
IPBC	153.0	76.5	38.3	19.1	12.2

4. Inject exactly 10.0 ul of each sample and collect data.

CALCULATIONS AND DATA PROCESSING

[SAMPLE CONC.] = {[INJECTION SOLUTION CONC.] X [A] X [B] X [C]}

INJECTION SOLUTION CONC. = from Quantitation Report, in ng/ul
A = Final Volume of Injection Solution, in ml.
B = Dilution Factors (if any).
C = for Effluent Samples = [1000]/[Volume Sample extracted - in ml]
= for Sediment Samples = [1]/[Dry Wt. Sample extracted - in g]

Reporting Units: for Effluent Samples = ug/l
: for Sediment Samples = ug/g

The concentration of the 3-Iodo-2-Propynylbutylcarbamate is entered in the laboratory database using "RENT" software and entering "IPBC" for the Results (Billing) Code.

contd. ...

REFERENCES

[1] Hewlett Packard HP1090 Liquid Chromatograph Fact File Handbook, First Edition - November 1987.
[2] Determination of % 3-Iodo-2-Propynylbutylcarbamate (IPBC) in Formulated Products (communication), Troy Chemicals, 88/08/23.
[3] Koppers' HPLC Method for Measurement of Low levels of 3-Iodo-2-Propynylbutylcarbamate (communication), Koppers Company, Inc., April 15, 1987.

REVISION HISTORY

Version 1.0 New method - November 1988

APPENDIX VII

Environment Canada method of DDAC analysis
(Still under development)

APPENDIX VIII

Environment Canada method for Resin Acids analysis

Environment Canada
Conservation and Protection

Laboratories
Pacific & Yukon Region

RESIN ACIDS V2.3 August, 1990 RA

Gas Chromatographic / Mass Spectrometric

SCOPE AND APPLICATION

This method is applicable to the analysis of any process or discharge in a pulp mill and sediments. The resin acids quantitativly determined in this test are 8(14)-Abietenic, Abietic, 12-Chlorodehydroabietic, 14-Chlorodehydroabietic, Dehydroabietic, Dichlorodehydroabietic, Dihydroisopimaric, Isopimaric, Levopimaric, Palustric, Pimaric, Sandaracopimaric and Neoabietic acids. The normal minimum detectable concentration using this procedure is 0.005 mg/l in effluents and 0.2 ug/g in sediments, although lower limits can be achieved using larger sample amounts than specified. Toxicity to fish from the standards MSDS (Material Safety Data Sheet) is given in the following table. The toxicity given is based on a 96-hour LC50 using rainbow trout, except Sandaracopimaric acid which is based on coho salmon.

RA COMMON NAME	SYNONYMS	TOXICITY
8(14)-Abietenic	13-alpha-H-delta-Dihydroabietic	toxic
Abietic	7,13-Abietadien-18-oic	0.7-1.5 mg/l
12-Chlorodehydroabietic	12-Chloro-8,11,13-Abietatrien-18-oic	0.6-0.9 mg/l
14-Chlorodehydroabietic	14-Chloro-8,11,13-Abietatrien-18-oic	0.6-0.9 mg/l
Dehydroabietic	8,11,13-Abietatrien-18-oic	0.8-1.7 mg/l
Dichlordehydroabietic	12,14-Dichloro-8,11,13-Abietatrien-18-oic	0.6-1.2 mg/l
Dihydroisopimaric	delta-7-Dihydroisopimaric	toxic
Isopimaric	---	0.4-1.0 mg/l
Levopimaric	8(14),12-Abietadien-18-oic	0.7-1.0 mg/l
Palustric	8-13-Abietadien-18-oic	0.5-0.6 mg/l
Pimaric	8(14),15-Pimaradien-18-oic	0.7-1.2 mg/l
Sandaracopimaric	---	0.4 mg/l
Neoabietic	---	0 6-0.7 mg/l

SAMPLE HANDLING AND PRESERVATION

The volume of the sample required for resin acid analysis depends on the amount of resin acid present which is dependent on the type of wood pulped and the process used to treat the wood. A one litre effluent sample or a 100 gram sediment sample is normally sufficient. Samples should be collected, without preservative, in heat treated glass bottles and stored at <4 C. Upon receipt at the lab they should be adjusted to pH 9 and stored at <4 C. Ideally the samples should be analyzed within 2 weeks but may be stored for considerably longer. The literature indicates that pulp mill effluent samples can be stored at 4 C for up to 30 days without any significant change in the concentration of any of the analytes of interest.

contd. ...

SUMMARY OF METHOD

Effluent samples are first adjusted to pH 9 and then extracted with diethyl ether. Sediments are extracted by shaking with methanol in a screw cap centrifuge bottle. The extracts are rotary evaporated and the resulting residues, containing the resin acids and other solvent soluble organics, are then redissoved in methanol, transferred to small vials and then methylated with diazomethane.

Methylation Reaction

$$\underset{\text{(Resin/Fatty Acids)}}{\text{R-COOH}} + \underset{\text{(Diazomethane)}}{\text{CH}_2\text{N}_2} \text{ -------> } \underset{\text{(Methyl ester)}}{\text{R-COOCH}_3} + \text{N}_2$$

The residue is re-dissolved in methyl t-butyl ether (MTBE) and injected on a High Resolution Gas Chromatograph / Low Resolution Mass Spectrometer (HRGC/LRMS) system for analysis. The chromatogram will show the methyl esters of the resin and fatty acids and other co-extracted compounds. The identification of the various resin acids is made on the basis of comparison of their absolute retention times to those of external standards, comparison of their relative retention times (RRT) to DHA and mass spectral matching techniques using the mass spectrometer quantitation programs and full ion spectral library search routines.

METHOD PERFORMANCE

1. DI water spiked with DHA to give a 1 mg/l solution produced 95.3% recovery over 8 replicates with a Standard Deviation of 1.30.
2. The linear coefficient of correlation for each resin acid standard was better than 0.998 (0.1 - 50 ug/ml solution with 2 ul injection).
3. Five sample replicates yielded 2.7 Average % Standard Deviation for DHA.

INTERFERENCES

1. The use of Total Ion Mode on the HRGC/LRMS system essentially eliminates potential interference problems by providing positive peak identification.
2. Any organic substance removed from the wood by the extraction process is a potential interference. Different interferences may be present depending on the treatment the wood has undergone before the effluent was discharged.
3. The use of equal portions of solvent and sample minimizes formation of any emulsion during the solvent extraction.
4. Any insoluble residue left on redissolving the evaporated extract in methyl t-butyl ether is probably lignin, which is more soluble in methanol.
5. Overlap between resin acid and fatty acid methyl esters is usually slight, but some minor fatty acids such as the C22, C24 and C26 saturated normal fatty acids potentially interfere with HRGC/FID determinations.

contd. ...

Interferences (ctd.)

6. Evidence exists in the literature that storage at low or high pH could lead to different results for some compounds. In particular, Neoabietic, Palustric and Levopimaric acids tend to undergo isomerization. Studies have shown they produce an equilibrium mixture consisting of 95% Abietic acid and 2-3% each of Neoabietic and Palustric acids under acidic (pH 2) conditions. At present it is deemed best to keep the samples cool (<4 C) during transport to the laboratory and adjust the pH to 9 upon receipt thus avoiding the obvious problems associated with accurate control of pH adjustment under field conditions.
7. The current literature indicates that standard solutions of resin acids are not stable in methanol but are stable in MTBE.
8. Levopimaric and Palustric acid co-elute, thus HRGC/LRMS is required to distinguish between these isomers. The available published information about their concentrations in pulp and paper mill effluents suggests that Levopimaric acid should be a minor component relative to Palustric acid. They can easily be distinguished by examination of their mass spectra. If a mixture is present then the amounts of each can be easily determined (even though the peaks overlap completely) by using the "MAXIMIZING MASSES PEAK PROFILER" on the ITD and then selection of the appropriate ions for quantitation; for Palustric acid - (301-302) and/or (241-242) amu; for Levopimaric acid - 146 amu.

SAFETY

The following is extracted from Aldrich Technical Information Bulletin #AL-113 concerning the generation of diazomethane:

CAUTION: USE ONLY THE GLASSWARE SUPPLIED WITH THE DIAZALD KIT. THE USE OF STANDARD GROUND GLASS JOINTS WILL CREATE AN EXTREME EXPLOSION HAZARD. DIAZOMETHANE MUST BE GENERATED ONLY IN AN EFFICIENT FUME HOOD AND BEHIND A SAFETY SHIELD. DIAZOMETHANE IS EXTREMELY TOXIC.

APPARATUS

All glassware must be washed with heavy-duty soap, rinsed with distilled water, rinsed with acetone and then heat-treated at 325 C for at least 12 hours prior to use. The glassware must be stored in an area protected from chemical contamination.

1. 100-ml graduated cylinders; 250-ml beakers; 250-ml separatory funnels; 500-ml round bottom flasks; 10-ml and 50-ml volumetric flasks.
2. Pasteur pipets, 7-ml glass vials with foil lined screw caps.

contd. ...

APPARATUS (ctd.)

3. Rotary evaporator.
4. Wrist-action shaker.
5. Screw top centrifuge tubes, 100-ml, with telon-lined caps.
6. Centrifuge.
7. Diazald kit, Aldrich #Z10,025-0.
8. Nitrogen solvent evaporation apparatus.
9. High Resolution Gas chromatograph / Low Resolution Mass spectrometer system (Perkin-Elmer 8320 GC / Perkin-Elmer (Finnigan 800) Ion Trap Detector) using an HP Ultra 2 column (HP, 25m x 0.20mm, 0.33u film thickness). The system uses splitless GC injection, split mode on the ITD POS and Helium venting. The computer system is a 10mhz Equity III+ with a 40 mb hard drive and is interfaced to a DEC PDP11/84 for data archiving. The ITD software is Version 4.1.

REAGENTS

1. Diethyl ether, pesticide grade, Burdick & Jackson.
2. Methyl t-butyl ether, pesticide grade, Burdick & Jackson.
3. Methanol, pesticide grade, Burdick & Jackson.
4. Ethanol, 95%.
5. Sodium hydroxide, ACS grade.
6. Sodium hydroxide solution, 0.5 N.
   - dissolve 2 grams NaOH, ACS grade, in 100 ml DI water.
7. Potassium hydroxide, ACS grade.
8. Sodium sulfate, anhydrous, ACS grade, heat treated at 325 C for 12 hrs.
9. Diazald (N-methyl-N-nitroso-p-toluenesulfonamide), Aldrich #D2,800-0.
10. Diazomethane, ethereal alcoholic solution.
    - glassware is assembled according to the instructions contained in Aldrich Technical Information Bulletin #AL-131.
    - diazomethane is generated from the Diazald reagent. The following is extracted from Aldrich Technical Information Bulletin #AL-113.

****

CAUTION

USE ONLY THE GLASSWARE SUPPLIED WITH THE DIAZALD KIT. THE USE OF STANDARD GROUND GLASS JOINTS WILL CREATE AN EXTREME EXPLOSION HAZARD.

DIAZOMETHANE MUST BE GENERATED ONLY IN AN EFFICIENT FUME HOOD AND BEHIND A SAFETY SHIELD.

****

contd. ...

REAGENTS (ctd.)

10. Diazomethane, ethereal alcoholic solution (ctd.).
    - a 250-ml distilling flask is fitted with a dropping funnel and an efficient condenser set for downwards distillation. The condenser is fitted to 2 receiving flasks in series, the second of which contains 30 ml of diethyl ether. The inlet tube of the second receiver dips below the surface of the ether and both receivers are cooled in a water-ice bath. Heat is supplied by a constant temperature water bath set to 65 C. The heated portion of the glassware is covered with aluminum foil to promote faster distillation.
    - using the 250-ml distilling flask dissolve 5 grams KOH in 8 ml DI water.
    - add 25 ml 95% ethanol.
    - add 21.4 grams (0.1 mole) Diazald to 200 ml diethyl ether, dissolve and add to the dropping funnel.
    - over a period of 25 minutes add this to the alkali solution. The rate of addition should equal the rate of distillation.
    - when the dropping funnel is empty slowly add another 40 ml of diethyl ether and continue distillation until the distillate is colourless.
    - the combined ethereal distillates contain about 3 grams of diazomethane.
    - store diazomethane over ether-washed NaOH in a glass bottle with a teflon top (NOT a ground glass top).
    - store the prepared diazomethane in an explosion proof freezer.
11. Dehydroabietic acid, 99+%, Helix Biotech Ltd. #R-001.
12. Abietic acid, 90-95%, Helix Biotech Ltd. #R-002.
13. Neoabietic acid, 99+%, Helix Biotech Ltd. #R-003.
14. Isopimaric acid, 99+%, Helix Biotech Ltd. #R-004.
15. Levopimaric acid, 99+%, Helix Biotech Ltd. #R-005.
16. Palustric acid, 90-95%, Helix Biotech Ltd. #R-006.
17. Sandaracopimaric acid, 85-90%, Helix Biotech Ltd. #R-007.
18. Chlorodehydroabietic acid, 85-95%, Helix Biotech Ltd. #R-008.
19. Dichlorodehydroabietic acid, 90-95%, Helix Biotech Ltd. #R-009.
20. Pimaric acid, 85-90%, Helix Biotech Ltd. #R-011.
21. Dihydroisopimaric acid, 99+%, Helix Biotech Ltd. #R-012.
22. 8(14)-Abietenic acid, 99+%, Helix Biotech Ltd. #R-013.
23. Methylated Mixed Resin Acid Stock and Levopimaric Acid Stock Solutions.

    - CAUTION: REACTIONS INVOLVING DIAZOMETHANE MUST BE CARRIED OUT IN AN EFFICIENT FUME HOOD. TOXIC AND EXPLOSION HAZARD.

    - weigh exactly to within 0.1 mg, 15-20 mg each of each resin acid standard (except Levopimaric acid) into a 7-ml heat-treated vial.
    - add 2 ml of methanol to the vial, dissolve, then VERY SLOWLY add the diazomethane solution until a strong yellow colour persists. Usually about 4 ml is required.
    - seal the vial and let stand in a fume hood for 30 minutes (the yellow colour should still persist).

contd ...

REAGENTS (ctd.)

23. Methylated Mixed Resin Acid Stock Solutions (ctd.).
    - open the vial and evaporate the solvent under a gentle stream of nitrogen to about 1 ml.
    - dilute to exactly 250 ml with methyl t-butyl ether.
    - if quantitation of Levopimaric acid is also desired then make up a separate stock solution containing only Levopimaric acid, as above.
23. Methylated Mixed Resin Acid Working Standards Solutions.
    - dilute the Mixed Resin Acid Standard Stock Solution as required to give the Working Standards Solutions listed below.
    - dilute the Levopimaric Acid Standard Stock Solution to give similar concentrations, labelling the mix "RALEV" instead, as required.

MIX #	SOURCE	DILUTION	DILUTION FACTOR	APPROX. CONC.
RAMIX-1	Stock Solution	no dil.	X 1	60 ug/ml
RAMIX-2	Stock Solution	5/10 ml	X 0.5	30 ug/ml
RAMIX-3	Stock Solution	2/10 ml	X 0.2	12 ug/ml
RAMIX-4	Stock Solution	1/10 ml	X 0.1	6 ug/ml
RAMIX-5	Stock Solution	0.5/10 ml	X 0.05	3 ug/ml
RAMIX-6	Stock Solution	0.1/10 ml	X 0.01	0.6 ug/ml
RAMIX-7	Stock Solution	0.02/10 ml	X 0.002	0.1 ug/ml

PROCEDURE - EFFLUENTS

1. Check that the sample pH is 9.0 +/- 0.1 with a pH meter. Adjust to within 0.1 pH unit, if required, with 0.5N sodium hydroxide or 0.5N sulfuric acid.
2. Add 100 ml of sample to a 250-ml separatory funnel and extract 3 times with 80 ml aliquots of diethyl ether.
3. Combine the extracts over anhydrous sodium sulfate for 30 minutes.
4. Transfer the treated extract, washing the sodium sulfate with 3 X 50 ml of diethyl ether, to a clean 500-ml round bottom flask.
5. Rotary evaporate the extract to near dryness using a water bath temperature of less than 35 C.
6. Dissolve the residue in 1 ml of methanol then transfer to a 7-ml vial. Carefully wash the flask with a further 1 ml portion of methanol, transfer, then repeat the wash step twice more (for a combined total of 4 ml).
7. SLOWLY add diazomethane solution to the vial until a strong yellow colour persists.
8. Seal the vial and let stand for 30 minutes in the fume hood. If the yellow colour of the diazomethane does not persist for the entire 30 minutes then evaporate the sample to small volume under nitrogen and repeat the methylation step, as required.

contd. ...

PROCEDURE - EFFLUENTS (ctd.)

9. Remove the cap and, under a gentle stream of nitrogen, evaporate the solution in the fume hood to very near dryness.
10. Dissolve, make up to exactly 5.00 ml with methyl t-butyl ether and proceed to PROCEDURE FOR GC/MS.

PROCEDURE - SEDIMENTS

1. Air dry about 15 grams of sediment to constant weight (usually overnight) in a fume hood.
2. Weigh 10 grams air-dried mixed sample into a 100-ml screw top centrifuge tube and add 60 ml of methanol.
3. Using a wrist action-shaker agitate the sample for 2 hours.
4. Centrifuge and decant the sample into a 500-ml round bottom flask.
5. Repeat the extraction twice more (1 hour each) and combine the extracts.
6. Treat the combined extracts with anhydrous sodium sulfate.
7. Rotary evaporate the treated extract to near dryness using a bath temperature of less than 35 C.
8. Dissolve the residue in 1 ml of methanol then transfer to a 7-ml vial. Carefully wash the flask with a further 1 ml portion of methanol, transfer, then repeat the wash step twice more (for a combined total of 4 ml).
9. SLOWLY add diazomethane solution to the vial until a strong yellow colour persists.
10. Seal the vial and let stand for 30 minutes in the fume hood. If the yellow colour of the diazomethane does not persist for the entire 30 minutes then evaporate the sample to small volume under nitrogen and repeat the above methylation step, as required.
11. Remove the cap and, under a gentle stream of nitrogen, evaporate the solution in the fume hood to very near dryness.
12. Dissolve, make up to exactly 5.00 ml with methyl t-butyl ether and proceed to PROCEDURE FOR GC/MS.

PROCEDURE FOR GC/MS

1. Set up the Perkin Elmer 8320 gas chromatograph as follows:

ISO TIME 1: 1.0 min
OVEN TEMP 1: 140 C
RAMP RATE: 4.0 C/min
OVEN TEMP 2: 280 C
ISO TIME 2: 15.0 min

CARRIER GAS: UHP Helium @ 35 psig

INJ TEMP: 280 C
DET TEMP: OFF
INJ MODE: Splitless
Relay 0 OFF -1.00 min
ON 1.50 min
LINEAR VEL: 40 cm/sec @ 140 C
35 cm/sec @ 280 C

contd. ...

PROCEDURE FOR GC/MS (ctd.)

2. Set the ITD transfer line temperature to 270 C and let the system equilibrate.
3. Check that the flow rate from the ITD transfer line splitter is 1.0 ml/min with the GC oven at 140 C.
4. Turn the ITD AGC "OFF" and check that the water and air background levels are within acceptable levels.
5. Check that the ITD RF response is linear and within acceptable levels.
6. Check that the ITD ZEROCHECK ADJUST is within acceptable levels.
7. Ensure that the Ion Trap Detector is tuned according to the ITD Operating Instructions manual and passes the ITD diagnostic tests. The automatic setup and tuning procedure normally provides satisfactory results.
8. Turn the ITD AGC "ON" and check that the AGC background is within acceptable levels.
9. Set the GC autosampler conditions as follows:

   FILL TIME: 7 sec
   FLUSH TIME: 7 sec
   INJECT TIME: 15 sec
   INJ./VIAL: 2

   Ensure the appropriate GC method and A/S program are entered into a SET in the AUTOMATION QUEUE (normally only this SET is active). Set the FLUSH FREQUENCY to 2 to make every second vial a methyl t-butyl ether flush vial. Always make the first sample a solvent blank.
10. Set the ITD ACQUISITION parameters as follows:

    ACQU MODE: Full Scan
    SCAN RANGE: 50-390 amu
    SCAN TIME: 1.000 secs
    THRESHOLD: 1 count
    AGC MODE: on
    MICRO-SCANS: 5
    FIL/MUL DELAY: 900 secs
    SCHED TIME: 37 minutes
    MASS DEFECT: 100 mmu/100 amu

    TUNE: Segment 1 Tuning # 75
    Segment 2 Tuning # 75
    Segment 3 Tuning # 100
    Segment 4 Tuning # 125
    BACKGROUND MASS: 49
    MS TEMPERATURES: Open Split: 270 C
    Xfer Line: 270 C
    Exit Nozzle: 270 C
    Manifold: 245 C

11. Create the appropriate ITD "ACQ" file for the autosampler. Ensure that the file names used do not conflict with existing files. Duplicate file names will cause the original file to be overwritten automatically if the ITD is in A/S mode. Note that duplicate injections will require different file names for each injection.
12. IMPORTANT NOTE - a quirk in the ITD software will partially reset the old ITD tuning values in effect at the time the "ACQ" file was created if an old "ACQ" file is recalled (i.e. if the system has been re-tuned and has a different EM voltage and "B" value, the software will erroneously use the old EM voltage value and the current sensitivity "B" value). Do not use old "ACQ" files as templates for new ones, rather create new "ACQ" files.
13. Start the A/S run from the ITD by a <CTRL><B> from the ACQUISITION page then issue a START QUEUE from the GC AUTOMATION CONTROL PAGE. The system will automatically inject and store data for the standards and/or samples.

contd. ...

CALCULATIONS AND DATA PROCESSING

1. Create a multilevel EXTERNAL STANDARD file using the RAMIX-2 Standard to create the file framework. Choose "Q" then "EXTERNAL STANDARD" from the CHROMATOGRAPH screen as per the Ion Trap Operating Instructions Manual. Name the file "RAMIXn" where "n" is a sequential number between 000 and 999 assigned as required.
2. Set the following general External Calibration File parameters:

   QUAN MASS: Total
   QUAN CALC: Quadratic 3 pnts
   SEARCH METHOD: Mass Spec
   SEARCH WINDOW: 2 secs
   FIT THRESHOLD: 700

3. Process the data files for each level of the RAMIX standards by selecting "Q" from the APPLICATIONS screen then "A" for AUTO QUANTITATION. Choose the appropriate standard data files using the "F4" menu key. Load the Calibration file framework created above using the "F5" key, ensure "APP CALI DAT" is turned on using <CTRL><C> then select "F2" for AUTOCALIBRATION. Do this for each calibration standard. Check calibration file linearity by selecting "Q", "C" then examine the CALIB REPORTS screens ("F1") and the CALIB PLOTS screens ("F2") for each compound in the calibration file. Modify parameters as required to obtain the most accurate results when the individual calibration data files are re-processed as sample files as a check.
4. Similarly, Levopimaric acid may be added to the CALIB file by analyzing the RALEV solutions. If mixtures of Leviopimaric and Palustric are present in a sample, as determined by a full library MS search then the calibration file will have to be modified for using the appropriate selected ions rather than the automatic full ion quantitation (see INTERFERENCES 6.).
5. To process the sample data files select "Q" from the APPLICATIONS screen, "A" for AUTO QUANTITATION, choose the appropriate data file using the "F4" key menu and the CALIBRATION file using the "F5" key menu. Ensure "APP QUAN DAT" mode is "OFF", <CTRL><A>; "INCLUDE NOT FOUNDS" mode is "ON", <CTRL><I>; and the "SINGLE STEP" mode is "ON", <CTRL><S> for semi-automatic integration (if desired) and then "F1" for AUTO QUANTITATION.
6. Quantitate the quantitation files by selecting "Q" from the main menu then "Q" for Quantitation Reports from the CALIBRATION MENU. Set the "OPTIONAL CALCULATION METHOD" mode "ON", <CTRL><C>; then set "ORIGIN POINT" mode "ON", <CTRL><O>. Calculate the INJECTION SOLUTION CONCENTRATIONS using "F2" then display and spool the reports. Do a full background corrected library search to confirm each peak the quantitation program has identified as a resin acid.

contd. ...

CALCULATIONS AND DATA PROCESSING (ctd.)

7. Typical RT´s (min:sec) and RRT´s to DHA are given below.

RESIN ACID	RT	RRT	RESIN ACID	RT	RRT
Pimaric	22:45	0.911	DHA	24:59	1.000
Sandaracopimaric	23:08	0.926	Abietic	25:55	1.037
Isopimaric	24:03	0.963	Neoabietic	27:07	1.085
Palustric	24:15	0.971	14-Chloro-DHA	28:58	1.160
Levopimaric	24:15	0.971	12-Chloro-DHA	29:40	1.188
8(14)-Abietenic	24:28	0.979	Dichlor-DHA	32:56	1.318
Dihydroisopimaric	24:38	0.986			

8. Using the Quantitation Report Injection Solution Concentration data calculate the Resin Acids concentration in the sample using the following:

[SAMPLE CONC.] = {[INJECTION SOLUTION CONC.] X [A] X [B] X [C]}

INJECTION SOLUTION CONC. = from Quantitation Report, in ug/ml
A = Final Volume of Injection Solution, in ml.
B = Dilution Factors (if any).
C = for Effluent Samples = [1]/[Volume Sample extracted - in ml]
= for Sediment Samples = [1]/[Dry Wt. Sample extracted - in grams]

Reporting Units = for Effluent Samples = mg/l
= for Sediment Samples = ug/g

9. The concentration of the Resin Acids is entered in the laboratory database by using "RENT" software and entering the following Package (Billing) Codes:

RESIN ACIDS: RA Procedure 850 (Effluents)
Procedure 851 (Sediments).

"RENT" will create the appropriate Results Codes automatically.

REFERENCES

[1] L. Nemeth, G. Tanner, A Study on Log-Conditioning Wastewater from Veneer Production in the B.C. Interior, EPS Technical Report (1972).
[2] I.H. Rogers, H.W. Mahood, The Analysis of Whole Kraft Effluents and Receiving Water Samples for Resin Acids by G.C., EPS Seminar, Vancouver, B.C., (1976).

contd. ...

REFERENCES (ctd.)

[3] Technical Bulletin No. 397, A Comparison of Results from the Analysis of Pulp Mill Effluents for Compounds of Pulpmill and Bleach Plant Origin. National Council of the Paper Industry for Air and Stream Improvement (NCASI), (1983).

[4] Technical Bulletin No. 501, Procedures for the Analysis of Resin and Fatty Acids in Pulp Mill Effluents. National Council of the Paper Industry for Air and Stream Improvement (NCASI), (1986).

[5] Analytical Methodology for Resin and Fatty Acids, M.O.E. (Ontario) M.I.S.A. Program, (1988).

[6] L. LaFleur, Procedure for the Analysis of Resin and Fatty Acids in Pulp Mill Effluents. National Council of the Paper Industry for Air and Stream Improvement (NCASI), (1986).

[7] Ion Trap Detector Operating Instructions Manual, Perkin-Elmer #0993-7173, (1986), Revised (1990).

REVISION HISTORY

Version 2.3	JUL/90	Additional Resin Acids added to package. Change to Rev. 4.1 on ITD software.
2.2	JUN/89	GC column change. Switch to narrow-bore ITD transfer line splitter, increase in ITD trap temperature.
2.1	SEP/88	Change to Rev. 4.00 on ITD software. Minor changes to accomodate GC A/S.
2.0	FEB/87	Change to GC/MS procedure. Samples now in methyl t-butyl ether. Neoabietic, Palustric and Levopimaric acids added. Change to pH 9 extraction.
1.3	MAY/87	Diazomethane now generated from Diazald. Extract acidified with 2N H_2SO_4, not conc.
1.2	JUL/86	Add Methanol to methylation step.
1.1	APR/86	Shift to high sensitivity range on FID/GC.
1.0	-	Original Method in 1979 manual.

contd. ...

RESIN ACID WORKSHEET

SAMPLE #____________________

METHOD VERSION _____________

SAMPLE VOLUME EXTRACTED =________ml

FINAL VOLUME in Me-t-butyl ether =______ml

A/S RUN YES NO

VOLUME INJECTED ____ul

REF #	RESIN ACID	DILUTIONS	INJ SOL CONC ug/ml	SAMPLE CONC mg/l
1	8(14)Abietenic			
2	Abietic			
3	12-Chloro-DHA			
4	14-Chloro-DHA			
5	Dehydroabietic			
6	Dichloro-DHA			
7	Dihydroisopimaric			
8	Isopimaric			
9	Levopimaric			
10	Neoabietic			
11	Palustric			
12	Pimaric			
13	Sandaracopimaric			

contd. ...

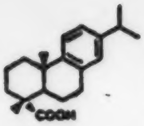
RESIN ACID WORKSHEET (ctd.)

P2/2

#	CALCULATIONS	
1	8(14)Abietenic	
2	Abietic	
3	12-Chloro-DHA	
4	14-Chloro-DHA	
5	Dehydroabietic	
6	Dichloro-DHA	
7	Dihydroisopimaric	
8	Isopimaric	
9	Levopimaric	
10	Neoabietic	
11	Palustric	
12	Pimaric	
13	Sandaracopimaric	

contd. ...

STRUCTURES of RESIN ACIDS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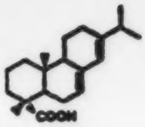
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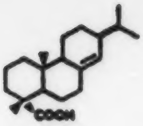
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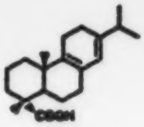
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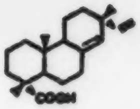
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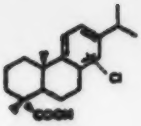
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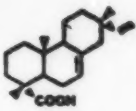
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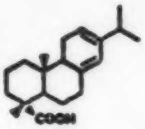
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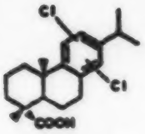
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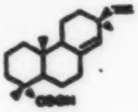
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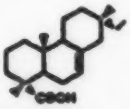
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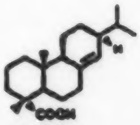

R-013

	COMMON NAME	SYNONYMS
R-001	Dehydroabietic	8,11,13-Abietatrien-18-oic
R-002	Abietic	7,13-Abietadien-18-oic
R-003	Neoabietic	---
R-004	Isopimaric	13b-Methyl-13-vinylpodocarp-7-ene-15-oic
R-005	Levopimaric	8(14),12-Abietadien-18-oic
R-006	Palustric	8-13-Abietadien-18-oic
R-007	Sandaracopimaric	---
R-008	12-Chloro-DHA	12-Chloro-8,11,13-Abietatrien-18-oic
R-008	14-Chloro-DHA	14-Chloro-8,11,13-Abietatrien-18-oic
R-009	Dichloro-DHA	12,14-Dichloro-8,11,13-Abietatrien-18-oic
R-011	Pimaric	8(14),15-Pimaradien-18-oic
R-012	Dihydroisopimaric	d-7-Dihydroisopimaric
R-013	8(14)-Abietenic	13-a-H-d-Dihydroabietic

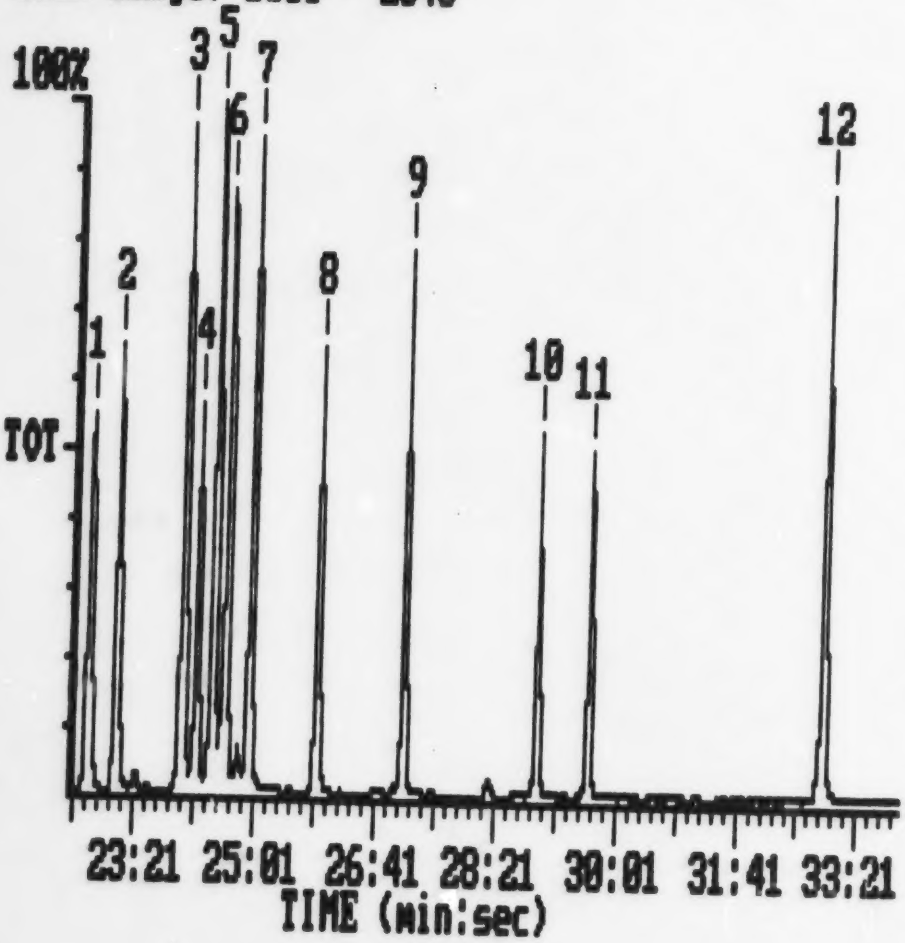
DHA=Dehydroabietic acid a=alpha b=beta d=delta

contd. ...

F.

CHROMATOGRAMS

Chromatogram: RESIN ACID STANDARD MIX
Comment: A/S INJECTION, 2 ul, ITD SOFTWARE V4.1, HP ULTRA-2 COLUMN
Scan Range: 1350 - 2040



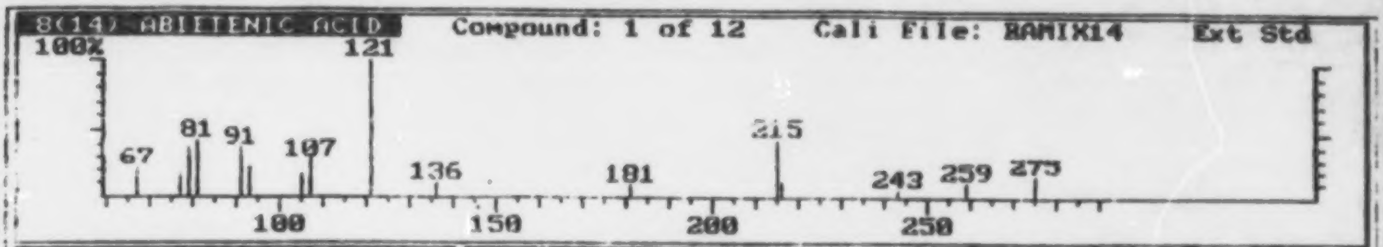
1 - METHYL PIMERATE
2 - METHYL SANDARACOPIMARATE
3 - METHYL ISOPIMARATE
4 - METHYL PALUSTRATE
5 - METHYL 8(14)-ABIETENATE
6 - METHYL DIHYDROISOPIMARATE
7 - METHYL DEHYDROABIETATE
8 - METHYL METHYL ABIETATE
9 - METHYL NEOABIETATE
10 - METHYL 14-CHLORO-DHA *
11 - METHYL 12-CHLORO-DHA *
12 - METHYL DICHLORO-DHA *

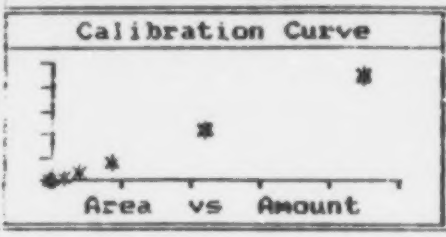
* - DHA = DEHYDROABIETATE

FILE

EXAMPLE OF ITD CALIBRATION

8(14) ABIETENIC ACID Compound: 1 of 12 Cali File: RAMIX14 Ext Std





Compound Statistics

```
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Search Window: 5 secs
Fit Threshold: [illegible]00
```
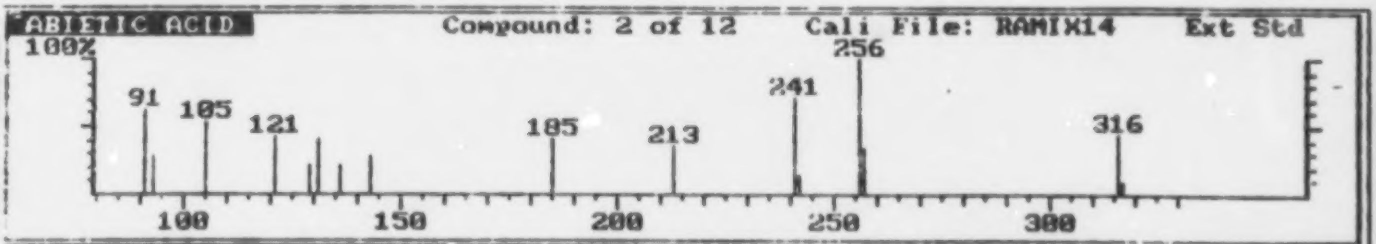
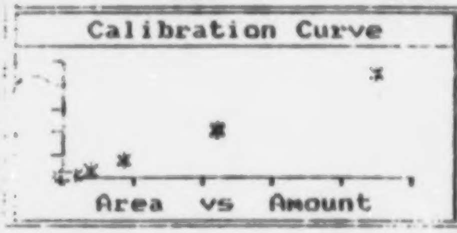

Mass/Int		Mass/Int	
67	75	121	336
77	68	136	39
79	126	181	35
81	149	215	155
91	127	216	38
93	75	243	19
105	59	259	36
107	96	275	57

ABIETIC ACID Compound: 2 of 12 Cali File: RAMIX14 Ext Std





Compound Statistics

```
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Quan Calc: Quad 3 pnts
Ret  Time: 25:53
Std Reference: 2
Search Method: Mass Spec
Search Window: 5 secs
Fit Threshold: [illegible]00
```
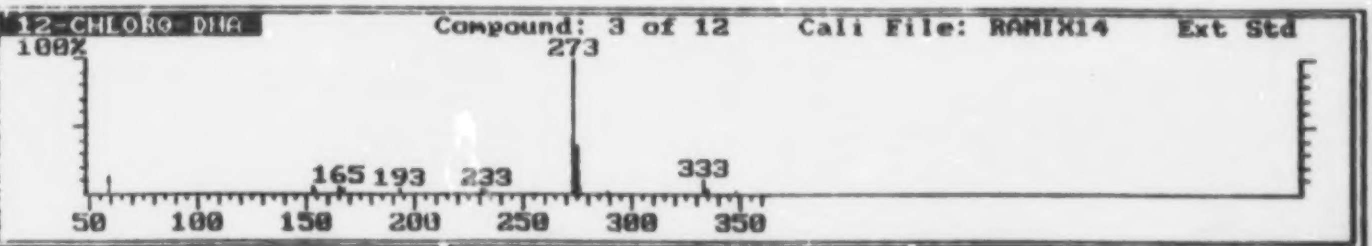
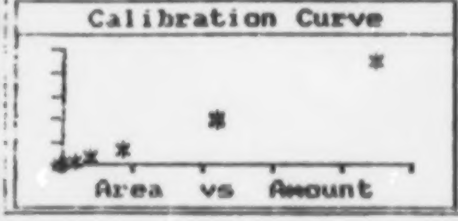

Mass/Int		Mass/Int	
91	122	185	82
93	56	213	71
105	105	241	139
121	84	242	29
129	42	256	194
131	82	257	66
136	43	316	89
143	56	317	18

12-CHLORO DHA Compound: 3 of 12 Cali File: RAMIX14 Ext Std





Compound Statistics

```
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Ret  Time: 29:3[illegible]
Std Reference: 3
Search Method: Mass Spec
Search Window: 5 secs
Fit Threshold: [illegible]00
```
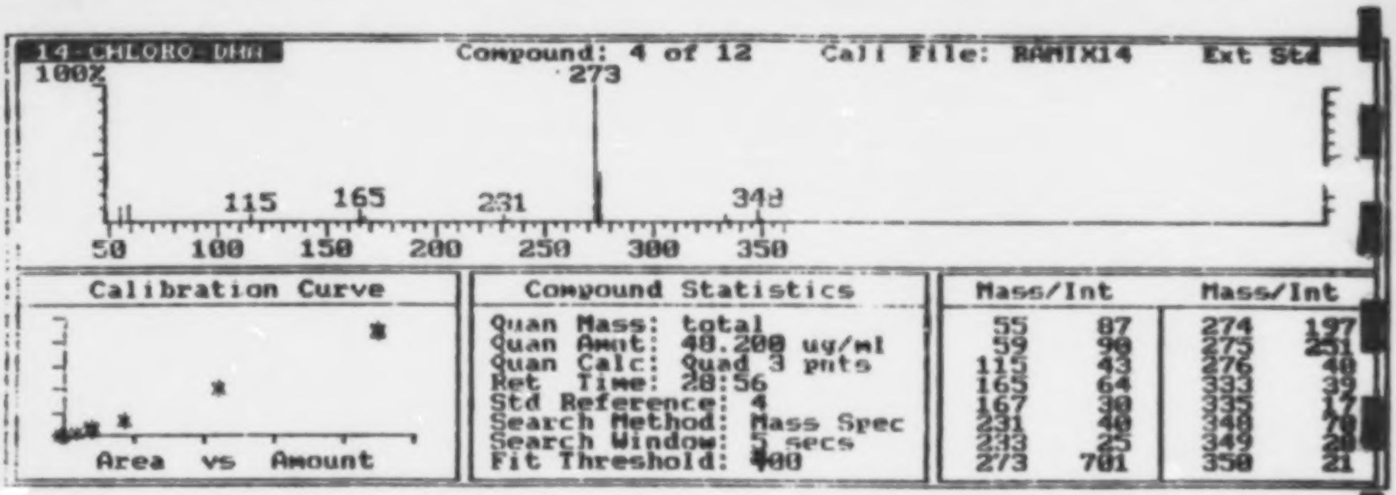

Mass/Int		Mass/Int	
59	101	273	694
153	45	274	196
154	[illegible]	275	[illegible]
165	47	276	[illegible]
167	[illegible]	[illegible]	[illegible]
193	[illegible]	333	77
231	[illegible]	335	[illegible]
233	28	348	22



```
14-CHLORO-DHA          Compound: 4 of 12    Cali File: RAMIX14    Ext Std

Compound Statistics                    Mass/Int        Mass/Int
Quan Mass: total                        55    87       274   197
Quan Amnt: 48.200 ug/ml                 59    90       275   251
Quan Calc: Quad 3 pnts                 115    43       276    40
Ret  Time: 28:56                       165    64       333    39
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Search Window: 5 secs                  233    25       349    20
Fit Threshold: 800                     273   701       350    21
```

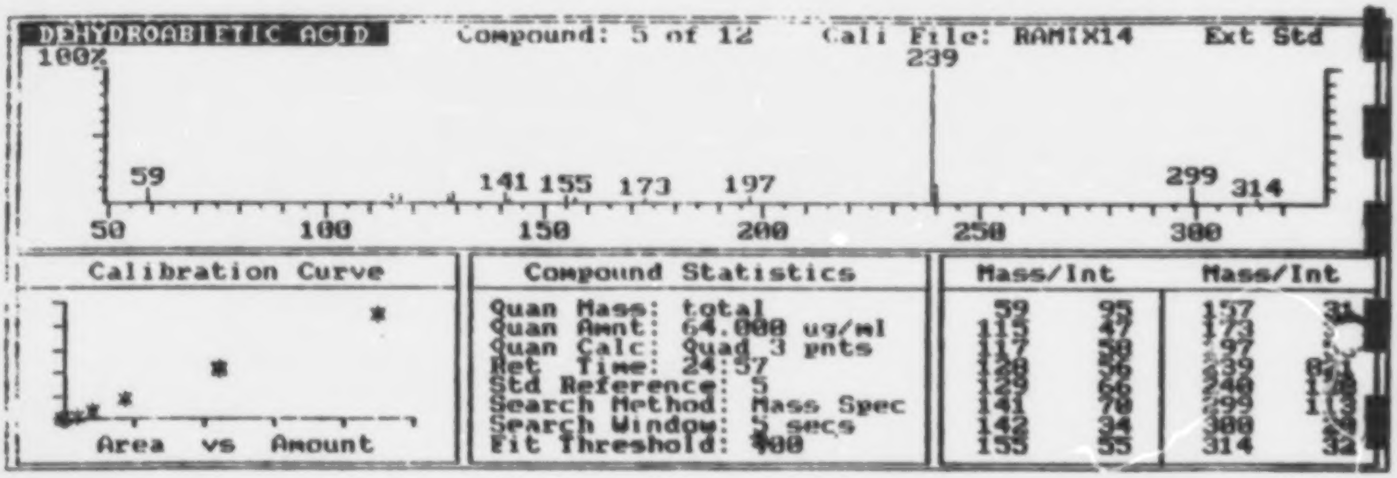

```
DEHYDROABIETIC ACID    Compound: 5 of 12    Cali File: RAMIX14    Ext Std

Compound Statistics                    Mass/Int        Mass/Int
Quan Mass: total                        59    95       157    31
Quan Amnt: 64.000 ug/ml                115    47       173   [illegible]
Quan Calc: Quad 3 pnts                 117    50       197   [illegible]
Ret  Time: 24:57                       128    56       239   [illegible]
Std Reference: 5                       129    66       240   [illegible]
Search Method: Mass Spec               141    70       299   113
Search Window: 5 secs                  142    34       300   [illegible]
Fit Threshold: 800                     155    55       314    32
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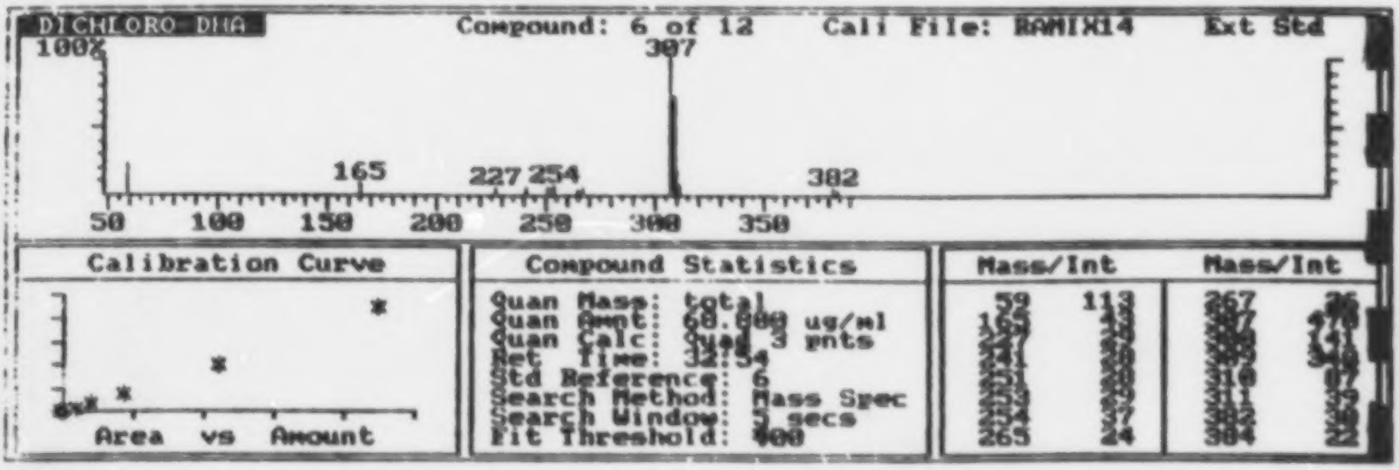
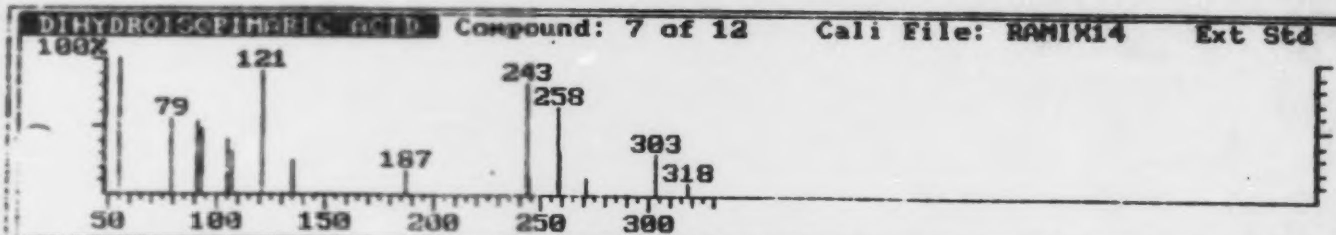


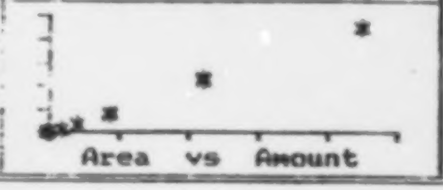
```
DICHLORO-DHA           Compound: 6 of 12    Cali File: RAMIX14    Ext Std

Compound Statistics                    Mass/Int        Mass/Int
Quan Mass: total                        59   113       267    26
Quan Amnt: 68.000 ug/ml                165   [illegible]   [illegible]   [illegible]
Quan Calc: Quad 3 pnts                 [illegible]   [illegible]   [illegible]   [illegible]
Ret  Time: 32:54                       [illegible]   [illegible]   [illegible]   [illegible]
Std Reference: 6                       [illegible]   [illegible]   310   [illegible]
Search Method: Mass Spec               [illegible]   [illegible]   [illegible]   [illegible]
Search Window: 5 secs                  254    37       382   [illegible]
Fit Threshold: 800                     265    24       384    22
```

DIHYDROISOPIMARIC ACID Compound: 7 of 12 Cali File: RAMIX14 Ext Std



Calibration Curve

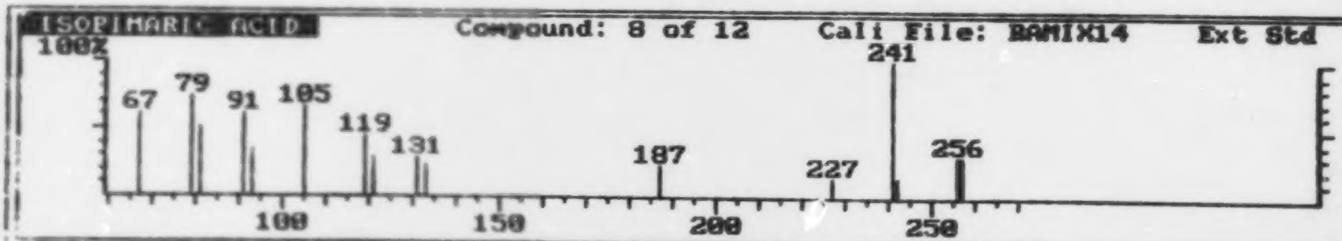


Compound Statistics

Quan Mass: total
Quan Amnt: 59.200 ug/ml
Quan Calc: Quad 3 pnts
Ret Time: 24:36
Std Reference: 7
Search Method: Mass Spec
Search Window: 5 secs
Fit Threshold: 900

Mass/Int		Mass/Int	
55	190	187	34
79	106	243	158
91	103	244	28
93	93	258	125
105	81	259	62
107	63	271	27
121	174	303	58
135	48	318	28

ISOPIMARIC ACID Compound: 8 of 12 Cali File: RAMIX14 Ext Std



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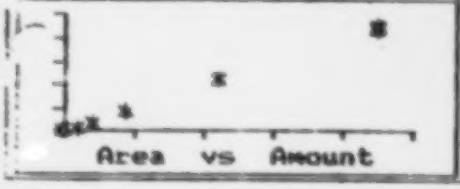
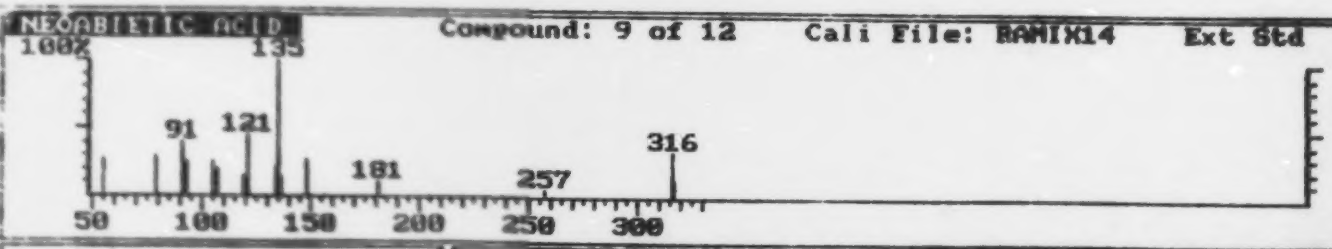


Compound Statistics

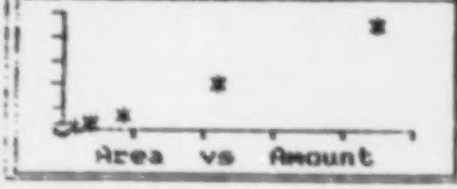
Quan Mass: total
Quan Amnt: 71.600 ug/ml
Quan Calc: Quad 3 pnts
Ret Time: 24:01
Std Reference: 8
Search Method: Mass Spec
Search Window: 5 secs
Fit Threshold: 900

Mass/Int		Mass/Int	
67	99	131	48
79	119	133	32
81	81	187	35
91	99	227	24
93	56	241	159
105	106	242	23
119	73	256	50
121	48	257	67

NEOABIETIC ACID Compound: 9 of 12 Cali File: RAMIX14 Ext Std



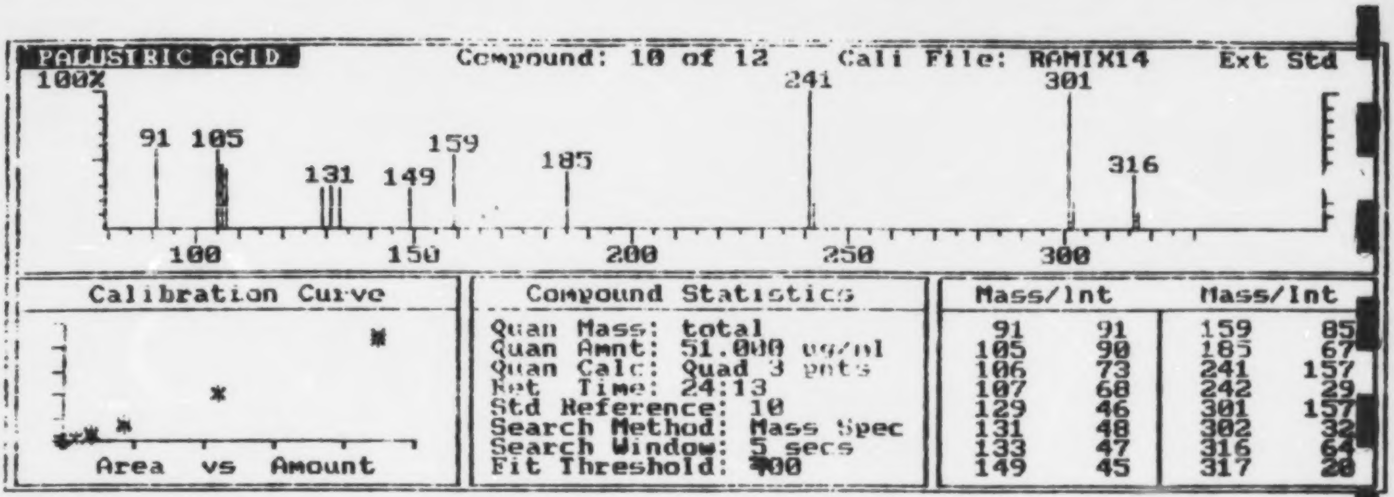
Calibration Curve



Compound Statistics

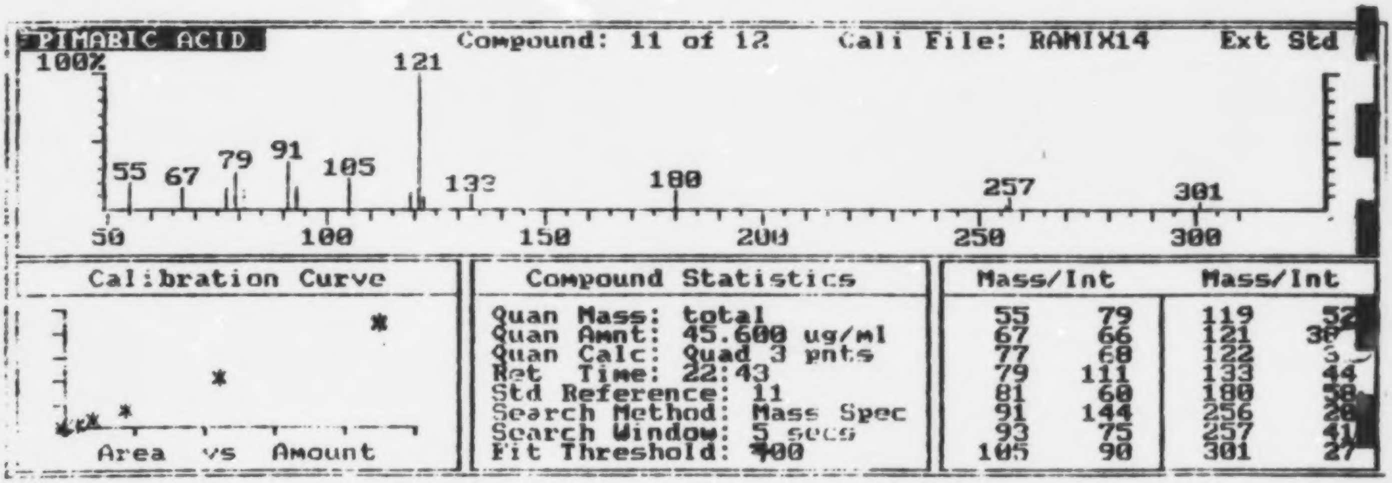
Quan Mass: total
Quan Amnt: 60.000 ug/ml
Quan Calc: Quad 3 pnts
Ret Time: 27:06
Std Reference: 9
Search Method: Mass Spec
Search Window: 5 secs
Fit Threshold: 900

Mass/Int		Mass/Int	
55	91	134	72
79	95	135	329
91	135	136	50
93	83	148	91
105	87	181	30
107	67	257	28
119	50	316	117
121	151	317	43



```
PALUSTRIC ACID       Compound: 10 of 12      Cali File: RAMIX14      Ext Std

Compound Statistics                     Mass/Int          Mass/Int
Quan Mass: total                         91    91          159    85
Quan Amnt: 51.000 ug/ml                 105    90          185    67
Quan Calc: Quad 3 pnts                  106    73          241   157
Ret  Time: 24:13                        107    68          242    29
Std Reference: 10                       129    46          301   157
Search Method: Mass Spec                131    48          302    32
Search Window: 5 secs                   133    47          316    64
Fit Threshold: 900                      149    45          317    20
```



```
PIMARIC ACID         Compound: 11 of 12      Cali File: RAMIX14      Ext Std

Compound Statistics                     Mass/Int          Mass/Int
Quan Mass: total                         55    79          119    52
Quan Amnt: 45.600 ug/ml                  67    66          121    30
Quan Calc: Quad 3 pnts                   77    68          122    3
Ret  Time: 22:43                         79   111          133    44
Std Reference: 11                        81    60          180    58
Search Method: Mass Spec                 91   144          256    20
Search Window: 5 secs                    93    75          257    41
Fit Threshold: 900                      105    90          301    27
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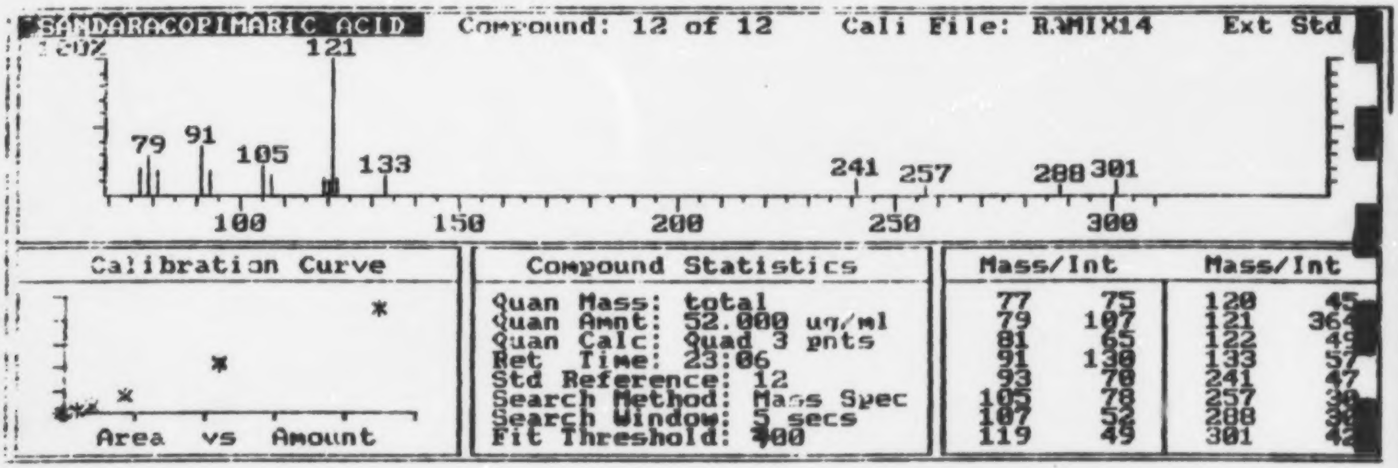

```
SANDARACOPIMARIC ACID  Compound: 12 of 12    Cali File: RAMIX14      Ext Std

Compound Statistics                     Mass/Int          Mass/Int
Quan Mass: total                         77    75          120    45
Quan Amnt: 52.000 ug/ml                  79   107          121   364
Quan Calc: Quad 3 pnts                   81    65          122    49
Ret  Time: 23:06                         91   130          133    57
Std Reference: 12                        93    70          241    47
Search Method: Mass Spec                105    78          257    30
Search Window: 5 secs                   107    52          288    3
Fit Threshold: 900                      119    49          301    42
```

APPENDIX IX

Provincial Guidelines and Laboratory Procedures for Measuring Acute Lethal Toxicity of Liquid Effluents to Fish

[illegible]

PROVINCIAL GUIDELINES AND LABORATORY PROCEDURES FOR MEASURING ACUTE LETHAL TOXICITY OF LIQUID EFFLUENTS TO FISH

Province of
British Columbia
Ministry of
Environment

Province of
British Columbia

MEMORANDUM

To: R. H. Ferguson,
Director,
Waste Management Branch.

Date: November 19, 1982.

File: SU 64.1205

Re: Standardization of Bioassay Procedures

Please find enclosed the final report of the bioassay task force. The report presents guidelines and laboratory procedures for fish bioassays. These procedures, which are patterned on the federal procedures, should produce consistent and comparable results for any one type of test measuring acute toxicity of effluents to fish. The document, therefore, meets the requirements of the Pollution Control Board which recommended the publication of the guidelines.

The selection of sample and bioassay type was studied by the task force and draft guidelines were produced. However, after reviewing comments from several agencies and organizations the task force decided that much more effort would be needed to produce acceptable guidelines, and therefore none were included in this report. Also, the subject of other aquatic bioassay methods, such as in situ tests, sub-lethal tests and tests employing organisms other than fish, was considered to be too far ranging to be included in the terms of reference. Any of these topics could be studied in future inquiries if you think that provincial guidelines are needed.

RJ Buchanan

R. J. Buchanan, Ph.D.,
Water Management Branch.

Encl.

MEMBERS OF THE BIOASSAY TASK FORCE

R.J. Rocchini (Chairman)	Aquatic Studies Branch, Ministry of Environment
J.A. Servizi	International Pacific Salmon Fisheries Commission
R.G. Watts	Environmental Protection Service, Environment Canada
S. Horvath	Environmental Laboratory, Ministry of Environment
M.J.R. Clark	Waste Management Branch, Ministry of Environment
H.J. Singleton	Aquatic Studies Branch, Ministry of Environment
R.H. Young	MacMillan Bloedel Ltd., for Council of Forest Industries of B.C.
A. Sholund	Imperial Oil Ltd., for British Columbia Petroleum Association
A.J. Jordan	Rescan Environmental Services Ltd., for Mining Association of British Columbia
D.J. McLeay	D. McLeay and Associates Ltd., for Council of Forest Industries of B.C., British Columbia Petroleum Association and Mining Association of British Columbia

PROVINCIAL GUIDELINES AND LABORATORY PROCEDURES FOR MEASURING ACUTE LETHAL TOXICITY OF LIQUID EFFLUENTS TO FISH

Report of the Bioassay Task Force

November, 1982

Canadian Cataloguing in Publication Data

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Provincial guidelines and laboratory procedures for measuring acute lethal toxicity of liquid effluents to fish

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ISBN 0-7719-9174-6

1. Fishes - Effect of water pollution on - Testing. 2. Water quality bioassay - British Columbia. 3. Toxicity testing - British Columbia. I. British Columbia. Waste Management Branch. II. Title.

SH174.B56 597'.024 C83-092104-4

SUMMARY

A task force was formed from government agencies and industry to draft guidelines and uniform laboratory procedures for fish bioassays throughout the Province. Use of these procedures and guidelines should produce consistent results for any one type of test used to monitor the acute toxicity of effluents to fish.

Procedures are given for holding and acclimating fish, transporting and storing effluent samples and conducting fish bioassays.

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1.0 INTRODUCTION

Following a public hearing in February, 1980, on pollution in the lower Fraser River, the Pollution Control Board concluded that guidelines and laboratory procedures for fish bioassays be established. Subsequently the Board recommended that the Waste Management Branch publish such guidelines and laboratory procedures, after consultation with interested parties.

The recommendation came about because certain results on acute toxicity of municipal effluents were not readily comparable due in part to variations in test procedures.

There are many methods of measuring acute toxicity, all of which have their application, but the use of similar laboratory procedures for fish bioassays throughout the Province will permit comparison of results for any one type of test. The purpose of the bioassay tests detailed here is to monitor the acute lethal toxicity of effluents to fish; that is to determine if diluted or undiluted effluent samples, tested under controlled laboratory conditions, cause fish mortalities.

The Waste Management Branch set up a task force with representatives from the Federal and Provincial Government and industry. The general terms of reference of the task force were as follows:

- To establish provincial guidelines and laboratory procedures for measuring the acute lethal toxicity of liquid effluents to fish.
- To specify effluent handling and sampling procedures.

In developing these procedures an attempt was made to balance scientific and practical considerations and to ensure that the results will be accurate and precise enough for the majority of situations in which they will be used.

Several aquatic bioassay methods are not included in this report as they are outside the terms of reference. These include, for example, in situ tests, sub-lethal tests and various tests employing organisms other than fish, such as algae, daphnia, or bioluminescent bacteria.

The report describes methods of effluent sampling and types of bioassays that can be performed, but it does not cover the selection of suitable sample and bioassay type for a given effluent. The selection of sample and bioassay type will depend on many factors, including the stability of the effluent and the variability of effluent toxicity. Also important are volume of effluent discharged, dilution in the receiving water, potential effects on water quality and aquatic life, cost of sampling and testing and site location and accessibility. Consideration of these factors could, if required, be the subject of a separate inquiry.

2. GENERAL PROCEDURES FOR FISH BIOASSAYS

General procedures for fish bioassays are based on the federal report: Standard Procedure for Testing the Acute Lethality of Liquid Effluents (1980). Some changes were made to the federal procedures to suit British Columbia conditions. It is believed that the use of the procedures given here will allow consistent and comparable bioassay results to be obtained for any one type of test.

This section lists laboratory test conditions which should be followed for all fish bioassays, whether static or flow-through. The listing is followed by a number of explanatory notes, which are intended to clarify certain points of the test conditions.

2.1 TEST CONDITIONS

2.1.1 Subject to item 2.1.14, rainbow trout (*Salmo gairdneri*) are to be used as the test species.

2.1.2 Only healthy stocks of fish acclimated as described in section 2.2.1, are to be used. Individual fish may be tested only once.

2.1.3 Individual fish are to have been actively feeding for a minimum of two weeks, and are to weigh between 0.2 and 5 grams. The length of the largest fish should not be more than two times the smallest in the same test.

2.1.4 A minimum of ten test fish are to be exposed to each test solution for a given time. At least ten control fish are to be exposed concurrently to the control water for at least the same time period.

2.1.5 The test is rendered invalid if fish mortality exceeds 10 percent in the control water.

2.1.6 For every one gram of fish, there should be at least 0.5 litre of test solution or control water for every 24 hours that the fish are exposed.

2.1.7 The minimum water depth in any test vessel should be 15 cm.

2.1.8 The test should be conducted at 15 ± 1°C.

2.1.9 Prior to the addition of fish, the control water and each test solution are to be pre-aerated, using a disposable glass pipette (not an airstone) in each test vessel, at no more than 7.5 mL $min^{-1}L^{-1}$, for not more than 120 minutes, to raise the dissolved oxygen to at least 60 percent saturation.

2.1.10 The test shall begin either after achieving at least 60 percent dissolved oxygen saturation within the maximum time limit (120 minutes) for aeration, or immediately after the maximum time limit is reached should 60 percent dissolved oxygen saturation not be achieved. If 60 percent dissolved oxygen saturation is not reached, the dissolved oxygen shall be measured at least twice in each 24-hour period during the test. Low dissolved oxygen may cause stress and contribute to observed mortalities.

2.1.11 An aeration rate of no more than 7.5 mL $min^{-1}L^{-1}$ can be applied to each test solution and control water throughout the test period.

2.1.12 Exclusive of aeration, no food or chemicals should be added to the test solutions or control water, except for pH neutralization if specified for compliance testing.

2.1.13 The total number of dead fish should be recorded for each test solution at least once each day, up to the completion of the test. Dead fish should be removed at least once each day. Fish are considered dead when they fail to respond to gentle prodding.

2.1.14 Effluents containing only freshwater or having a salinity of less than or equal to 10 parts per thousand whether discharged into freshwater or seawater should be tested with rainbow trout acclimated to freshwater. Effluents containing seawater and/or brine and having a salinity greater than 10 parts per thousand and deposited into seawater should be tested with an appropriate fish species acclimated to seawater of similar salinity to that of the effluent. Effluents containing brine and deposited into freshwater should be tested with rainbow trout acclimated to freshwater.

2.1.15 When the effluent sample must be transported or stored, the sample should be kept in sealed non-toxic containers excluding any air. The sample should be stored in darkness and tested as soon as possible, but no more than five days after collection. For effluent sample types shown to be inherently unstable, a shorter time between collection and testing is advised and should be specified for compliance testing. Alternatively, on-site testing may be considered. To evaluate effluent stability the results of static tests performed on a grab sample immediately after collection and after four days of storage should be compared. If after four days the toxicity, expressed in toxic units*, changes by 50 percent or more, the effluent could be classed as highly unstable.

2.1.16 The effluent sample may be kept at ambient temperature for 48 hours after collection, except in the case of extreme ambient temperature during transport and storage. Samples should be maintained at about 4°C thereafter, except for those portions adjusted to the bioassay temperature within 24 hours of testing. For effluent sample types shown to be inherently unstable, as described in section 2.1.15, temperature control may be required immediately after collection, and should be specified for compliance testing. Alternatively, on-site testing may be considered.

2.2 EXPLANATORY NOTES

2.2.1 HOLDING AND ACCLIMATION OF FISH

Fish should be acclimated to the following laboratory conditions over a period of at least two weeks prior to testing. The purpose of acclimation is to determine that fish are healthy and to allow them to adjust to the holding conditions. A record should be kept of water quality, fish disease and mortality during acclimation and holding.

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*toxic units $= \dfrac{100}{\text{96h LC50}}$, where 96h LC50 is in volume percent

(Esvelt _et_ _al_., 1973).

a) Water Quality

Holding, diluent and control water can be receiving water, or water from another source having alkalinity, hardness and nonfilterable residue no greater than the annual mean in the receiving water. It is recommended that water quality be monitored according to the characteristics and frequency in Table 1.

b) Holding Facilities

The acclimation tanks and accessories must be made of non-toxic materials, such as glass, porcelain, fibreglass, stainless steel, polyethylene, acrylic, polypropylene or fibreglass-reinforced polyester. Copper, galvanized material, natural rubber, brass and lead must not come in contact with holding, acclimation or dilution water, or with effluent samples or test solutions. The acclimation tanks should be located away from any physical disturbances and preferably in a location separate from the test vessels.

During acclimation, a constant flow of water through the holding tanks is necessary. Rates of water exchange should be a minimum of 1.4L/g of fish per day. To prevent overcrowding there should be at least one litre of water for every 10 g of fish in the holding tank (Sprague, 1973).

TABLE 1

RECOMMENDED PARAMETERS AND SAMPLING FREQUENCY FOR DILUENT AND CONTROL WATERS

Parameter	Frequency		
	Monthly	Twice Yearly (Spring and Fall)	Source Dependent
Total ammonia nitrogen	✓		
Specific conductance	✓		
Dissolved oxygen	✓		
Hardness	✓		
Nonfilterable residue	✓		
pH	✓		
Residual chlorine			✓
Salinity			✓
Temperature	✓		
Total alkalinity	✓		
Total organic carbon	✓		
Total organophosphorus pesticides			✓
Total organophosphorus pesticides plus polychlorinated biphenyls			✓
Total: aluminum, arsenic, calcium, cadmium, cobalt, chromium, copper, iron, mercury, potassium, magnesium, manganese, sodium, nickel, lead and zinc		✓	

c) Photoperiod

The photoperiod for acclimation and testing should be either a constant sequence of 14 hours of light and 10 hours of darkness or the natural photoperiod. It is recommended that lights be turned on or off gradually over at least 15 minutes.

d) Aeration in Holding Tanks

Supplementary aeration by filtered, oil-free compressed air should be provided if necessary to keep the dissolved oxygen level at greater than 80 percent saturation.

e) Cleaning of Holding Tanks

Holding tanks should be kept clean. Designs for tanks which are partially self-cleaning are available. However, periodic siphoning of settled material is usually necessary. Tanks should also be disinfected between batches of fish to minimize the occurrence of disease.

f) Temperature

Since the temperature of the holding water used during the transfer of fish from the hatchery to the laboratory may be outside of the acceptable limits for the test (15 ± 1°C), it may be necessary to gradually change the temperature until the desired temperature for acclimation is achieved. The fish should be held at this temperature for a 14-day acclimation period before being used in a bioassay.

g) Feeding

Fish shall be fed a recognized commercial fish food suitable for rainbow trout. The mean condition factor K for rainbow trout ($K = \frac{100w}{\ell^3}$) at the time of testing shall fall in the range 0.7 to 1.3, where w is the wet weight in grams and ℓ the fork length in centimetres. Fish shall not be fed for at least 12 hours prior to testing.

h) Disease Detection and Control

Daily inspection of fish in holding tanks is an essential part of the detection of disease, as "healthy" versus "unhealthy" conditions should be recognized (Roberts and Shephard, 1974). Fish should be disease-free, and observed mortalities during acclimation and holding should be less than 1 percent per week.

2.2.2 TEST AND CONTROL VESSELS

Glass is the preferred material for test and control vessels. Vessels, as well as measurement devices shall be thoroughly cleaned and rinsed in accordance with good laboratory practice.

2.2.3 START OF TEST

Prior to commencing the bioassay the test vessels and accompanying tubing should be rinsed with control water. Test concentrations for 96h LC50 determinations may be an appropriate series of five or more. To facilitate the calculation of bioassay results, for example the interpolated values for LC50 tests, it is recommended that a test concentration series be chosen from a logarithmic table such as Table 2. The aliquots of effluent used in the test must be representative of the total effluent sample available. The aliquots must be thoroughly mixed immediately before introduction into the test vessels. Temperature, dissolved oxygen levels and pH should be recorded for each test vessel and the fish randomly introduced.

2.2.4 SPECIAL CONSIDERATIONS FOR CERTAIN TYPES OF SAMPLE

If the effluent sample contains a high level of settleable solids, these should be re-dispersed throughout the test. The re-circulation of diluted or undiluted suspensions through test vessels with vertical sides and steeply sloped conical-shaped bottoms is recommended for this purpose (Noggle, 1978). Samples in the collection containers must be agitated thoroughly just prior to pouring to ensure the re-suspension of all solids.

TABLE 2

SERIES OF EFFLUENT CONCENTRATIONS FOR USE IN 96h LC50 TESTS

Column (Number of Concentrations Between 10 and 1)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
3.2	4.6	5.6	6.3	6.8	7.2	7.5	7.7	7.9	8.1	8.2	8.4	8.5	8.6	8.7
1.0	2.2	3.2	4.0	4.6	5.2	5.6	6.0	6.3	6.6	6.8	7.0	7.2	7.3	7.5
	1.0	1.8	2.5	3.2	3.7	4.2	4.6	5.0	5.3	5.6	5.9	6.1	6.3	6.5
		1.0	1.6	2.2	2.7	3.2	3.6	4.0	4.3	4.6	4.9	5.2	5.4	5.6
			1.0	1.5	1.9	2.4	2.8	3.2	3.5	3.8	4.1	4.4	4.6	4.9
				1.0	1.4	1.8	2.1	2.5	2.8	3.2	3.5	3.7	4.0	4.2
					1.0	1.3	1.7	2.0	2.3	2.6	2.9	3.2	3.4	3.6
						1.0	1.3	1.6	1.9	2.1	2.4	2.7	2.9	3.2
							1.0	1.3	1.5	1.8	2.0	2.3	2.5	2.7
								1.0	1.2	1.5	1.7	1.9	2.2	2.4
									1.0	1.2	1.4	1.6	1.8	2.0
										1.0	1.2	1.4	1.6	1.8
											1.0	1.2	1.4	1.5
												1.0	1.2	1.3
													1.0	1.2
														1.0

Midpoints between Concentrations in Column X will be found in Column (2X+1).
The listed values can represent concentrations expressed as percent by volume or weight, or milligrams per litre or parts per million (ppm) by weight or volume.
Values may be multiplied or divided, as necessary, by any power of 10.

An example of table use and subsequent LC50 determination is as follows:-

> A toxic sample was estimated to have a 96h LC50 value near 1% concentration by volume. From Column 4, the following series of five concentrations were selected to include and bracket the estimated LC50 value; 2.5%, 1.6%, 1.0%, 0.63% and 0.4% (the last two values were Column 4 listings divided by 10). The bioassay provided the following observations of cumulative fish mortality after 96 hours of exposure; 100% mortality in concentrations of 2.5%, 1.6% and 1.0%, 0% mortality in concentrations of 0.63% and 0.4%. The resulting 96h LC50 value of 0.79% was determined from column 9 (2x column 4+1) of the table and confirmed by a semilogarithmic plot of the % concentration versus the % mortality data.

For further information on the use of such a table see the Environment Laboratory Manual, 1979.

Test suspensions should be continuously drawn from the bottom of the cone and pumped onto the surface of the suspension. Fish within these test vessels are to be held in soft mesh baskets for protection from pumps and to permit daily inspection for mortalities. The level of suspended solids in each test vessel should be measured upon introduction of fish, and at 24-hour intervals thereafter.

If the effluent sample contains a high level of floatable material (ie. oil or surfactants), test suspensions should be agitated throughout the test to ensure mixing and exposure of fish to soluble constituents. The re-circulating conical vessels described above may be used for this purpose. Alternatively, cylindrical test vessels with individual impellers should be used to maintain homogeneous emulsions (EPS 1973, Blackman et al., 1978). Fish must be protected from impellers by rigid-frame cages.

For all highly colored or opaque effluent samples, or for samples producing foam in the test vessel, fish are to be inspected at least daily by raising them to the surface in a suitable basket, or by carefully drawing a dip net through the vessel.

2.2.5 OTHER OBSERVATIONS

Measurements should be made daily of the aeration rate, temperature, pH and dissolved oxygen of each bioassay test solution and of the control water.

2.2.6 BIOASSAY RECORD SHEETS

The information shown in Table 3 should be recorded on bioassay record sheets.

TABLE 3

INFORMATION TO BE RECORDED ON BIOASSAY INFORMATION RECORD SHEETS

A. Laboratory details:
- Laboratory identification
- Tester(s)

B. Sample details:
- Sample type (grab, composite, on-line)
- Sample source
- Sample description
- Collection date and time
- Storage/shipping details
- Date and time received in laboratory

C. Control/Diluent Water details:
- Water type
- Water source (date, time collected if not tap water)
- Current values for parameters listed in TABLE 1.

D. Test Organism details:
- Species
- Source
- Treatments
- Acclimation details
- Food type and quantity fed
- Feeding schedule
- Average length, weight, and condition factor, with ranges
- Holding temperature
- Photoperiod

E. Test details:
- Test type performed (special apparatus if used)
- Exposure duration
- Exposure dates
- Photoperiod
- Aeration details, preaeration details
- Replacement/flow rate
- Test temperature
- Test depth
- Test volume, test vessel description
- Number of test organisms per test vessel
- Loading rate/day
- Concentrations established (w/w, v/v, active ingredient)

F. Test Observation details:
- Effects, mortalities/test concentration/observation time
- pH, D.O., temperature/test concentration/observation time

G. Test Result details:
- Limit/LT50/LC50 determined value with method
- 95% confidence limits & statistical procedures used

H. Other Appropriate details.

3. SPECIFIC PROCEDURES FOR FISH BIOASSAYS

This section defines different bioassay procedures recommended for measuring the acute lethal toxicity of effluents to fish. The test conditions described in section 2 apply to all these procedures. This section also defines the types of effluent sample which may be used for bioassays and the measurements used to express acute lethal toxicity.

3.1 DESCRIPTION OF SPECIFIC BIOASSAY PROCEDURES

3.1.1 STATIC BIOASSAY

In the static technique, the fish are exposed to the same test and control solutions for the duration of the test. This test may employ either grab or composite effluent samples as described in section 3.2.

3.1.2 STATIC REPLACEMENT BIOASSAY

In the static replacement technique, test fish are exposed to fresh test solution every 24 hours for the duration of the test by replacing the test solution. Control fish are treated similarly.

The replacement of solution may be carried out either by transferring the fish from one test vessel to another, or by renewing at least 90 percent of the test solution in the original test vessel. Control water is replaced similarly. This test may employ either grab or composite effluent samples as described in section 3.2.

3.1.3 FLOW THROUGH BIOASSAY

In the flow through technique, test solution flows continually through the test vessel, at a rate of at least 2 litres per gram of fish per day for the duration of the test. To ensure adequate replacement of solution the total flow during any six-hour period should be equal to or greater than the volume of test solution in the test vessel. Control fish are subject to the same conditions using control water.

This test may employ either a grab or a composite effluent sample as described in section 3.2. The sample flows continually from a storage tank using metering and dilution equipment of a type such as described in Standard Methods (1980).

3.1.4 ON-LINE BIOASSAY

In this technique, test solution flows continually through the test vessel in the manner and at the rate described for the flow through bioassay (section 3.1.3). However, rather than using a grab or composite effluent sample, effluent is conveyed directly and continuously from the effluent source to the metering and dilution system and hence to the test vessel. This test must therefore be performed at the site where the effluent originates.

3.2 DESCRIPTION OF EFFLUENT SAMPLE TYPES

3.2.1 GRAB SAMPLE

The grab sample is a single or discrete sample of the effluent collected in a period not exceeding 15 minutes where practical. In some cases sequential grab samples may be specified to supply effluent for static replacement or flow through bioassays.

3.2.2 COMPOSITE SAMPLE

a) Simple Composite Sample

The simple composite sample is composed of a series of equal aliquots of effluent collected at least hourly over a total time period of 24 hours or as related to the periodicity of the effluent discharge. The collection of the aliquots may be intermittent or continuous. Continuous collection requires a constant flow of effluent to the sample containers. The total series of aliquots collected comprising a single composite must be thoroughly mixed in a single container before use. In some cases simple composite samples may be collected sequentially to supply effluent for static replacement or flow through bioassays.

b) Flow Proportional Composite Sample

The flow proportional composite sample is composed of a series of aliquots of effluent collected at intervals of time so that the rate at which each aliquot is collected is proportional to the rate at which the effluent is being discharged. The total collection time should be 24 hours or as related to the periodicity of the effluent discharge. The collection of the aliquots may be continuous or intermittent. Continuous collection requires a continuous flow of effluent to the sample containers. For intermittent collection aliquots must be collected at least once per hour. The total series of aliquots collected comprising a single composite must be thoroughly mixed in a single container before use. In some cases flow proportional composite samples may be collected sequentially to supply effluent for static replacement or flow through bioassays.

3.3 DESCRIPTION OF MEASUREMENTS FOR EXPRESSING ACUTE LETHAL TOXICITY

3.3.1 LIMIT BIOASSAY

The limit bioassay is used to determine if the toxicity of a sample is above or below a specified value. This test requires the survival of 50 percent or more of the test fish over 96 hours, in an effluent concentration equal to the specified value. The test may therefore require the use of only one effluent concentration plus a control.

3.3.2 LT50

The LT50 is the calculated lethal time to death of 50 percent of the test fish at a specified concentration of the effluent. The LT50 test should be continued for 96 hours for surviving fish. The 95 percent confidence limits for the LT50 values should be calculated.

3.3.3 96h LC50

The 96h LC50 is the calculated concentration of effluent which is lethal to 50 percent of the test fish during a 96-hour exposure. In setting up the 96h LC50, at least five different concentrations of effluent in dilution water plus a control should be used. To calculate the 96h LC50 from the test data, the methods described by Standard Methods (1980) may be used. The 95 percent confidence limits should be calculated for 96h LC50 values.

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